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EXPERIMENTAL ARTICLES

A Laboratory Model of the Cyanobacterial Mat from the Kotel'nikovskii Hot Spring (Baikal Region)

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Abstract—A laboratory model of the cyanobacterial community of the Kotel'nikovskii hot spring (Baikal Region) was developed. A step-by-step description of the algocenosis formation along both the time and temperature gradient was given. The natural and laboratory mats were compared, and the major differences in the qualitative and quantitative composition of the cyanobacterial community were revealed. The laboratory algocenosis was stratified by species composition and characterized by rapid replacement of the dominant cyanobacterial species depending on the temperature gradient. The formation of the community structure occurred during the 18 days of the experiment. In space and time, the sequence of species emergence in the cyanobacterial mat was as follows: *Mastigocladus laminosus* ⟶ *Phormidium tenue* ⟶ *Ph. ambiguum* ⟶ *Ph. valderiae*. The species composition of the laboratory mat was similar to that of the natural mat; however it was found to be less diverse.

Key words: hot spring, community, cyanobacterial mat, laboratory model, temperature gradient.

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Due to the unique adaptive capabilities of the microbial communities of hot springs, they have attracted attention of the researchers for a long time [1, 2]. Moreover, these consortia are considered to be analogues of the most ancient biocenoses on the Earth [3].

The formation, development, and functioning of hydrothermal cyanobacterial communities can be studied using laboratory models, which develop along the temperature gradient in the form of biofilms (mats) analogous to the natural ones [4].

The advantage of laboratory models is that they allow much more detailed information on the community development obtained under the conditions of a controlled experiment. In addition, obtaining of a laboratory community may be considered an intermediate stage of microbial culture isolation. The development of laboratory models of microbial communities involves certain difficulties in both selection of the optimal growth conditions and preservation of cell viability.

Previous studies of the Kotel'nikovskii hot spring (Baikal Region) were focused on in situ investigations of microbial mats: their structure, species diversity, and community functioning were investigated [5–7].

The aim of the present work was to obtain a cyanobacterial community from the Kotel'nikovskii hot spring during a controlled experiment and to compare the laboratory and natural communities.

MATERIALS AND METHODS

Water and cyanobacterial mat samples were collected in the summers of 2003 and 2004 from the Kotel'nikovskii hot spring, located on the northwestern shore of Lake Baikal. Water discharges from the spring well with a flow rate of 4 l/s and forms a stream. The water is of the fluoride–hydrocarbonate–sodium type with a mineralization of 0.2 g/l, pH 9.4; the highest water temperature is 81°C [8, 9]. The samples were collected at four stations with different water temperatures.

To determine the species composition, samples of cyanobacterial mats were fixed with 4% formalin; prior to inoculation, unfixed samples were stored at 4°C. The water temperature in the spring was measured with a Prima electric sensor thermometer (Portugal). Cyanobacterial species were identified on the basis of their morphology determined by light microscopy (Axiostar plus; Carl Zeiss, Germany) at ×1000 magnification; the manuals were used [10, 11]. Cyanobacterial cells were enumerated in a Goryaev count chamber [15] in ten replicates. The proportion of each species was calculated as a percentage of the total number of cyanobacterial cells in the community.

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In order to cultivate a cyanobacterial community under laboratory conditions, a continuous-flow tray-type unit equipped with a heater was used [4] (Fig. 1). The unit included a slightly sloping rectangular tray (51 × 5 cm). The flow rate of the medium was 5 ml/min. The community was cultivated on a medium for thermophiles containing the following (g/l): $CaCl_2$, 0.05; $MagSO_4 \cdot 7H_2O$, 0.1; NaCl, 0.008; $NaNO_3$, 0.6; K_2HPO_4, 0.1; $FeCl_3$, 0.028; $NaHCO_3$, 0.1; Na_2SiO_3, 0.2; and trace element solution. At the early stages of the experiment, pre-homogenized mat samples were placed into the unit. The inlet and outlet temperatures of the medium were 66 and 31°C, respectively. The intensity of 24-hour illumination was 2000 lx. The duration of the experiment was 30 days; the samples were collected every three days. For the description and observations, a total of seven reference points were marked in the tray at regular intervals (Table 1).

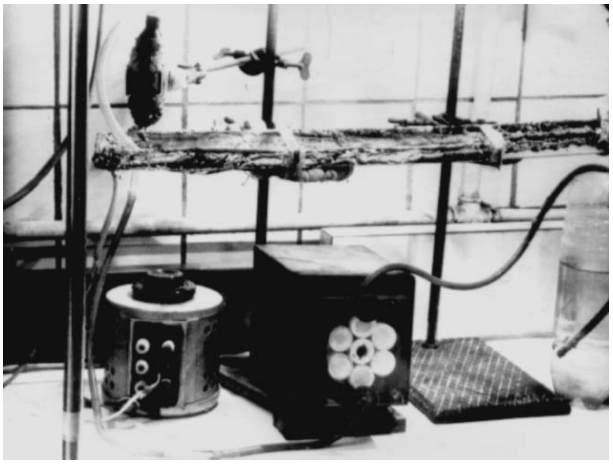

Fig. 1. The continuous-flow tray-type unit used to obtain the laboratory model of the cyanobacterial mat.

RESULTS AND DISCUSSION

The Structure of the Natural Community

Near the spring outlet (69–82°C), no cyanobacterial mats were detected. Along the edge of the hot spring (5 m from the outlet) where temperature was lower (45–50°C), local development of black leatherlike mats up to 3 mm thick was observed. *Mastigocladus laminosus* was the main and almost the only component of cyanobacterial mats. This species is common in high-temperature habitats and can be found in almost all hot springs around the world [11]. At the same time, in the hot springs of Buryatia, this species had not been previously detected by other authors [5–7, 12–14, 16, 19]. Low numbers of *Phormidium* filaments remnants were also detected at this point.

Two types of thin (up to 1.5 mm) mats (green and reddish-brown, with black patches) were observed at 33–36°C. In these mats, *M. laminosus* and *Ph. angustissimum* were predominant; low numbers of *Ph. tenue* were detected as well. Filaments of *Oscillatoria limosa*, *O. proboscidea*, and *O. simplicissima* formed the black patches on the cyanobacterial mats; filaments of *Phormidium* were scarce.

At 25–30°C, a brilliant green, layered cyanobacterial mat up to 1.5 mm thick developed. This mat was distinguished by the maximum number of cyanobacterial species typical of this spring. Representatives of the species *Ph. tenue* and *Ph. valderiae* prevailed. The following species were often found: *Ph. angustissimum*, *O. limosa*, *O. proboscidea*, *O. simplicissima*, and *Calothrix* sp. Representatives of *Gloeocapsa* sp. and *Ph. ambiguum*, as well as the filamentous green bacterium *Chloroflexus aurantiacus* and diatoms, were scarce. The characteristic trait of this mat was its mosaic structure: along with green patches, black ones were observed, in which the total number of *Oscillatoria* exceeded the amount of the elsewhere predominant members of *Phormidium.*

A total of ten cyanobacterial species were detected in the investigated ecosystem. As the distance from the spring outlet increased, a shift in the predominant cyanobacterial species occurred as follows: *M. laminosus* ⟶ *M. laminosus* + *Ph. angustissimum* ⟶ *Ph. tenue* + *Ph. valderiae.* For some generic taxa, confinement to certain temperature zones was observed. For instance, *Mastigocladus* was detected only at high temperatures (45–50°C), whereas species of the genus *Phormidium* grew within a broad temperature range (25–50°C); species of the genus *Oscillatoria* grew at low temperatures (25–30°C).

Table 1. Properties of the reference points of the cyanobacterial mat grown in the tray

Reference points	1	2	3	4	5	6	7
Distance from the medium inflow point, cm	3	10	17	24	31	38	45
Temperature, °C	66	48	41	36	33	32	31
Thickness of the medium layer, cm	0.1	0.5	0.7	0.9	1.0	1.2	1.5

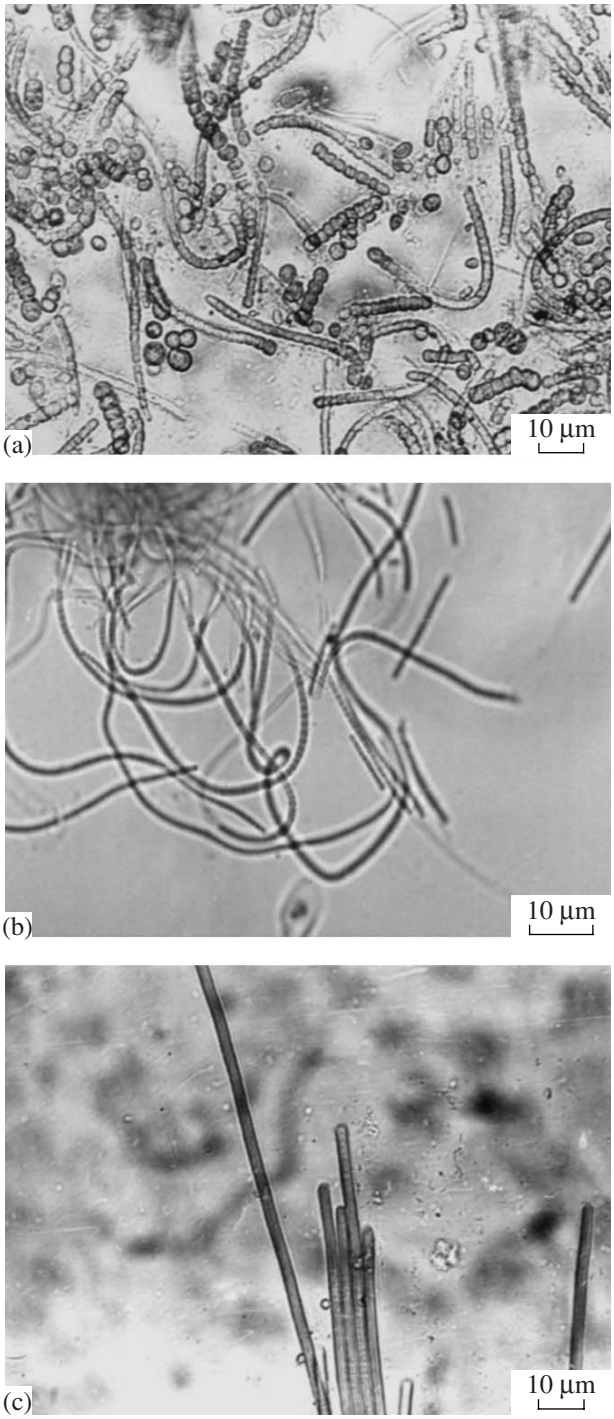

Fig. 2. View of the laboratory community at (a) 48°C (*M. laminosus* prevails); (b) 36°C (*Ph. tenue* prevails); and (c) 32°C (*Ph. ambiguum* prevails).

Previous investigations of the Kotel'nikovskii hot spring and its cyanobacterial mats did not reveal such species as *M. laminosus, Ph. valderiae*, and *Ph. angustissimum*; at the same time, *Ph. purpurascens* and *Synechococcus* sp. (which we had previously detected in the present study) were observed [5, 7].

Thus, in the studied hot spring, drastic changes in the species composition of the community, as well as in its qualitative and quantitative dynamics, occurred with decreasing temperature. To every temperature range, there is a corresponding community type with its own qualitative and quantitative composition, which is typical for most communities developing across the temperature gradient [5, 16–19].

Description of the laboratory model

The formation of the cyanobacterial mat under laboratory conditions involved several stages. Observations of this process allowed us to determine the strategies of growth and substrate colonization of cyanobacteria.

At the first stages of the experiment (1–3 days), after the unit was supplemented with a homogenized cyanobacterial suspension, the formation of cell aggregates was observed, followed by the aggregate separation and sedimentation of cells. The largest amount of the aggregated cell suspension was observed at points 2 to 5. At point 1, characterized by a high temperature and the low level of the medium, growth was not detected. By day 3, a layered biofilm of brilliant green color consisting was formed on the bottom and walls of the tray between points 3 and 7; it consisted of two species of cyanobacteria, *M. laminosus* and *Ph. tenue*. The first one was predominant in the biofilm formed between points 3 and 6; the second one prevailed at point 7.

By day 6, a tendency towards spatial differentiation of cyanobacterial species was observed. The film has ceased to be homogeneous. Acrobryous colonies, in which *M. laminosus* prevailed, were detected. However, *Ph. tenue* was found to be dominant in the biofilm. The final separation of species in the community did not occur at this stage, and the species developed together at all the points.

By day 9 of the experiment, the biofilm was 1–3 mm thick and distributed across the whole tray, including point 2. Between points 2 and 4, the biofilm began to divide into two layers, the surface and subsurface ones. In the latter one, acrobryous colonies (*M. laminosus*) grew.

By day 12, intense growth of the biofilm began at point 2; *M. laminosus* was the major component in both the surface and subsurface layers of the biofilm. In the surface layer, the proportion of cells of the dominant species was up to 100% of the total number of cyanobacterial cells, whereas in the subsurface one it was up to 70%. At point 4, *M. laminosus* prevailed in the surface colonies; in the subsurface layer, *M. laminosus* and *Ph. tenue* were codominants. At this point, *Ph. ambiguum* was detected for the first time, whereas *Ph. valderiae* was first revealed at point 6. In addition to the latter,

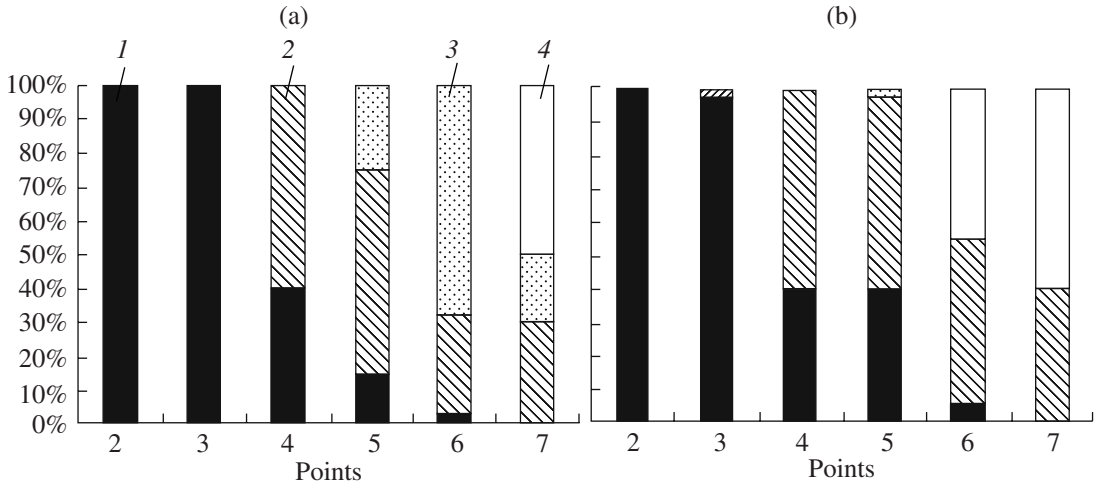

Fig. 3. Distribution of cyanobacterial species along the temperature gradient under laboratory conditions: (a) surface biofilm and (b) subsurface biofilm. *1*, *M. laminosus*; *2*, *Ph. tenue; 3, Ph. ambiguum; 4, Ph. valderiae*.

Ph. tenue, which formed the basis of the surface and subsurface layers of the biofilm, was detected at this point.

By day 15 of the experiment, the biofilm became looser; its thickness reached 3–5 mm across the whole tray. By day 18, the final distribution of species along the temperature gradient occurred in the mature cyanobacterial mat. It depended on the biofilm location (surface or subsurface) (Figs. 2, 3).

At point 2 (48°C), only *M. laminosus* occurred in the surface and subsurface layers. A similar pattern was observed at point 3 (41°C); however, *Ph. tenue* (2%) was detected in the subsurface layer. At point 4 (36°C), a replacement of the dominant species occurred. The proportion of *M. laminosus* decreased to 40%, while *Ph. tenue* became the major forming component, both at the surface and subsurface.

At point 5 (33°C), *Ph. tenue* remained the predominant species. The proportion of *M. laminosus* in the surface biofilm decreased further, to 15%. *Ph. ambiguum* cells appeared at this point for the first time. Their amount varied from 2% at the bottom to 25 % at the surface.

At point 6 (32°C), the prevalence of species depended on the biofilm spatial arrangement: *Phormidium tenue* was predominant at the bottom, whereas *Ph. ambiguum* prevailed at the surface. In addition to the above-mentioned species, cells of *Ph. valderiae* appeared. Their amount in the subsurface biofilm rapidly reached significant values (up to 45%). The proportion of *M. laminosus* cells was 2–5% of the total number of cyanobacteria in the community.

At point 7 (31°C), similar to the previous point, four cyanobacterial species were present at the same time. At this point, predominance of *Ph. valderiae* both in the surface and subsurface biofilms (50–60%) was observed for the first time. The specific weight of *Ph. tenue* cells was still considerable (30–40%). Trichomes of *M. laminosus* were scarce.

No further changes in the qualitative and quantitative composition of the algocenosis occurred until the end of the experiment; solidification and a slight thickening of the cyanobacterial mat were observed.

As a result of the successful selection of the cultivation conditions, a laboratory model of the cyanobacterial mat from the Kotel'nikovskii hot spring was obtained. Formation of the community structure under the specified conditions occurred during the first 18 days of the experiment.

At different stages (along the time gradient) of the mat formation and in different temperature zones (along the temperature gradient), the numbers of species in the community increased. The mature model algocenosis consisted of four cyanobacterial species. In space and time, the species appeared in the cyanobacterial mat in the following sequence: *M. laminosus* $\longrightarrow$ *Ph. tenue* $\longrightarrow$ *Ph. ambiguum* $\longrightarrow$ *Ph. valderiae*; this order was the same in all experiments. Each species, at its initial appearance in the community, immediately replaced the preceding dominant.

All these patterns were typical of both the surface and subsurface biofilms. All the above-mentioned species appeared simultaneously in both the surface and subsurface biofilms. The proportion of *M. laminosus* cells was highest at high temperatures and decreased gradually as temperatures decreased. This species grows well under laboratory conditions and was found to be thermophilic [2, 4]. Starting at point 4 (36°C), the proportion of *Ph. tenue* was significant (30–60%) in both biofilms. At the same time, in the subsurface biofilm this species was detected at higher temperatures than in the surface. *Ph. ambiguum* grew only at the surface; its trichomes in the subsurface biofilm were

Table 2. Diversity of cyanobacteria in the natural and laboratory communities along the temperature gradient

Temperature, °C	Natural conditions				Laboratory conditions						
	69–82	45–50	33–36	25–30	66	48	41	36	33	32	31
Species											
Mastigocladus laminosus Cohn.		+	+	+		+	+	+	+	+	+
Phormidium tenue (Menegh.) Gom.			+	+			+	+	+	+	+
Ph. ambiguum Gom.				+					+	+	+
Ph. valderiae (Delp.) Geitl.				+						+	+
Ph. angustissimum W. et G.S. West		+	+	+							
Oscillatoria limosa Ag.				+							
O. proboscidea Gom.				+							
O. simplicissima Gom.				+							
Calothrix sp.				+							
Gloeocapsa sp.				+							

scarce. It may be suggested that this species prevails at high illumination intensity.

Hence, the obtained laboratory model of the cyanobacterial mat was an algal system stratified in the composition and ratio of cyanobacterial species along the time and temperature gradients.

Comparison of the Laboratory and Natural Communities

At a temperature higher than 50°C, cyanobacterial mats did not form both under natural and laboratory conditions. An increase in the species diversity which occurred as the temperature decreased may be considered as supporting evidence that high temperatures inhibited cyanobacterial growth (Table 2). These changes were most pronounced in the natural community at temperatures above 36°C. For instance, only three cyanobacterial species were detected at 33–36°C, whereas, at 25–30°C, ten species were present. The increase in the species diversity of the laboratory algocenosis was successive; in each temperature zone, the diversity increased by one species unit only. Within the low temperature range of 25–33°C, all types of the studied communities were observed. The fact that a slight decrease in temperature between the reference points resulted in changes in the species composition of the community is a characteristic trait of the low-temperature zone of the cyanobacterial mat grown under laboratory conditions. It is likely that both the temperature and spatial factors affect the formation of the laboratory community.

In the laboratory algocenosis, the species distribution along the temperature gradient was similar to that occurring in nature. Both in the natural and laboratory communities, the distribution of certain cyanobacterial species was confined to certain temperature zones. *M. laminosus* was the only species that grew at high temperatures; its proportion was significantly lower at low temperatures both under natural and laboratory conditions. During our in vitro and in situ experiments, at the lowest temperatures (25–33°C), *Ph. ambiguum* and *Ph. valderiae* were most numerous species. *Ph. tenue* was characterized by its predominance in the medium-temperature zone (33–36°C); at the interface between the low-temperature and medium-temperature zones its proportion remained significant.

The diversity of cyanobacterial species under natural conditions was greater: a total of five genera and ten species were detected, whereas only two genera and

four species were revealed in the community grown under laboratory conditions. It is obviously impossible to reproduce all the aspects of the natural conditions with complete precision in the laboratory and to consider all the factors which may affect cyanobacterial development. Some species that were detected in the natural community therefore showed no growth under laboratory conditions. At the same time, the community that grew under laboratory conditions included only those species that could be found in nature. Similar patterns were previously observed in laboratory models of cyanobacterial mats of the outlets of the hot springs of the Uzon caldera [4]. Representatives of the genera *Oscillatoria, Calothrix*, and *Gloeocapsa* were absent from the community developed under artificial conditions. The comparative study of the natural and laboratory mats revealed another difference: one thermophilic species was replaced with another one. *Ph. angustissimum*, which grew and even prevailed in the natural community, was not detected in the cyanobacterial mat grown under laboratory conditions, whereas *Ph. ambiguum*, whose cells were scarce in the natural community, showed good growth under laboratory conditions. Here, within the limits of one genus, a small-cell form was replaced by a large-cell one.

Under natural conditions, a shift in the predominant cyanobacterial species along the temperature gradient occurred as follows: *M. laminosus* ⟶ *M. laminosus*+ *Ph. angustissimum* ⟶ *Ph. tenue* + *Ph. valderiae.* Under laboratory conditions, however, it occurred according to the following scheme: *M. laminosus* ⟶ *Ph. tenue* ⟶ *Ph. ambiguum* ⟶ *Ph. valderiae.* The natural community was characterized by the presence of the dominant complex, whereas individual species were predominant in the laboratory community. The predominant forms were similar in both studied communities. The laboratory community differed from the natural one in that the naturally dominant *Ph. angustissimum* was replaced by *Ph. ambiguum.*

Thus, we developed a laboratory model of the cyanobacterial community from the Kotel'nikovskii hot spring (Baikal Region), stratified by the species composition and characterized by a rapid replacement of the dominant cyanobacterial species depending on the temperature gradient. The species composition of the laboratory mat was similar to that of the natural mat; it was, however, found to be less diverse. The obtained laboratory community may be considered a reliable analogue of the natural one and serve as an object for studies of the ecology and physiology of its components.

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