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EXPERIMENTAL ARTICLES

Investigation of Bacterioplankton in the Loess-containing Lake Khanka

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Abstract—Some characteristics of bacterioplankton—generation time, daily (*P*) and specific (*P/B*) bacterioplankton production, and bacterial metabolic coefficient K_2—in the loess-containing Lake Khanka were determined using five modifications of the bacterial-count procedure with the fluorescent dyes fluorescamin and erythrosin. Experiments showed that the organomineral complex (OMC) in this lake is broken down by chemoorganoheterotrophic bacteria. The increase in the loess content of the lake water intensified bacterial growth and the cycles of potassium, silicon, and other biogenic elements. The addition of starch to a loess suspension activated the breakdown of OMC due to the adsorption of starch on the OMC/water interface and stimulation of the metabolism of attached bacteria.

Key words: bacterioplankton, methods of bacterial count, bacterial production, heterotrophs, chemoorganoheterotrophs, organomineral complex.

The biogeochemical activity of microorganisms in water bodies considerably depends on the ecological, physical, and chemical characteristics of the environment. To correctly appreciate this activity in a water body, one has to evaluate not only the microbial population but also the ecological status of the water body and the intensity of trophic and metabolic processes in it [1, 2].

The bottom sediments of Lake Khanka are formed by clay and sandy aleurites. Due to the shallow depth of Lake Khanka (no more than 6 m), continuous wave motion agitates these deposits and impedes their settling on the bottom. As a result, the lake water contains up to 154 mg/l of loess particles during warm periods [3, 4].

To the best of our knowledge, information available in the literature on microbial activity in Lake Khanka is limited to the fragmentary data of our previous investigations [3, 5, 6].

The present work was undertaken to study the composition, productivity, and role of bacterioplankton in the organic-matter cycle in the loess-containing Lake Khanka.

MATERIALS AND METHODS

Bacteria were counted under an ML-2B luminescence microscope operated at a magnification of 1000×. The number of microscopic fields examined was chosen so that the variation coefficient did not exceed 10%. Namely, this coefficient ranged from 2.2 to 5.4% when bacteria were counted on filters by examining 15–20 microscopic fields and from 2.9 to 5.5% when bacteria were counted on glass slides by examining 70–80 microscopic fields.

Bacteria present on detritus and inorganic terrigenous particles can be enumerated most correctly by epifluorescence microscopy [2, 7]. This method makes it possible to detect even very small bacterial cells by their bright luminescence against the dark background.

In our experiments, we used 0.17-μm-pore-size filters, whose intrinsic fluorescence was suppressed by treating them with Sudan black B. Bacteria, were counted by five slightly modified methods. In method I, bacterial cells present in water samples were first stained with fluorescamin and then passed through the filters. Cells adsorbed on the filters could be desorbed by adding borate buffer, pH 9. In method II, samples were first filtered and then cells remaining on the filters were stained with fluorescamin. In method III, 0.2-ml aliquots of water samples were uniformly distributed over the 400-mm^2 area of thoroughly degreased microscopic slides, fixed in the alcohol vapor, dried, and the smears thus prepared were stained with fluorescamin in borate buffer. Methods IV and V were the same as methods II and III, respectively, except that erythrosin was used instead of fluorescamin for staining.

The bacterial biomass was estimated with allowance for the average volume of bacterial cells and their shrinkage. The average volume of bacterial cells in Lake Khanka, which was determined by the known formulas for sphere (cocci) and cylinder (rod-shaped cells), was 0.54 μm^3; the shrinkage coefficient was taken to be 1.6 [2, 7–9]. The generation time and daily

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Table 1. Bacterioplankton population (M) as estimated by different staining procedures

Method	Staining procedure	M, million cells/ml	δ	m	n
I	Staining with fluorescamin before filtration	5.02	0.77	0.22	6
II	Staining with fluorescamin after filtration	4.39	1.24	0.50	6
III	Fluorescamin staining of bacterial smears on slides	4.21	0.71	0.35	6
IV	Staining with erythrosin after filtration	2.59	1.52	0.62	6
V	Erythrosin staining of bacterial smears on slides	4.12	1.23	0.61	6

bacterial production were estimated from changes in the number of bacteria over a certain time period in two water samples. In one of these samples, from which bacterial grazers were removed by filtration through 2.8-μm-pore-size filters, bacteria only reproduced, while in the other sample, which was not filtered, bacteria not only reproduced but also could be eaten [2].

Bacteria of different physiological groups were counted by plating water samples onto standard selective media. Heterotrophic bacteria were detected on the medium described in the handbook [10]; nitrogen-fixing bacteria were detected on a solid Winogradsky medium; ammonia- and nitrite-oxidizing bacteria were detected in liquid Winogradsky media; and denitrifiers were detected on an Omelyanskii medium (nutrient agar containing 0.1% potassium nitrate and bromthymol blue [10]). Pikovskaya medium was used to detect the microorganisms that convert almost insoluble calcium triphosphate into phosphates soluble in water or diluted acids [10]; Zak medium was used to detect bacteria capable of converting potassium aluminosilicates into the water-soluble forms of potassium, aluminum, and silicon [10].

Chemical elements were analyzed by standard techniques [11, 12].

In separate experiments, we evaluated the ability of bacteria from Lake Khanka to accumulate various chemical elements and to grow in media containing different amounts of lake water.

The water of Lake Khanka was sampled in September 1997 at the following stations: st. 113 situated in the coastal zone of the lake 1 km from the southwestern shore, st. 72 situated in the Komissarovka River 2 km upstream from the mouth; st. 68 situated in the First Erik River mouth; and sts. 107 and 114 situated in the southwestern part of the lake [5].

RESULTS AND DISCUSSION

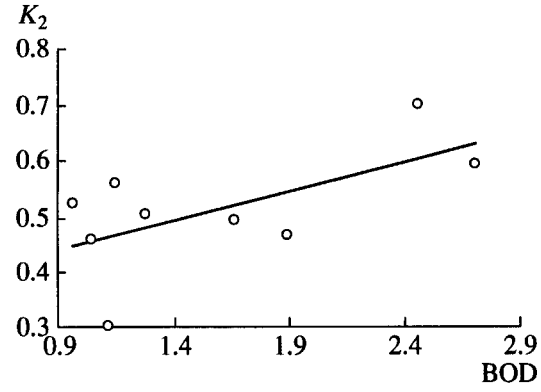
An important point in studying a lacustrine microflora is the choice of adequate methods for the quantitative evaluation of bacterioplankton. In our study, five variants of bacterial count in samples taken at st. 113 (three samples), st. 68 (two samples), and st. 72 (one sample) (Table 1) were used. Comparing the results of bacterial count using methods in which cells were stained with fluorescamin on slides and filters showed that the difference between these results was insignificant (calculated $t_{st} = 1.50$ versus the tabular value $t_{st} = 2.45$). At the same time, the results of bacterial counts by methods in which cells were stained with erythrosin and fluorescamin on filters considerably differed (calculated $t_{st} = 3.68$ versus the tabular value $t_{st} = 2.45$). In the final analysis, method I proved to be most adequate for the total bacterial count, while the other four methods underestimated the number of bacterial cells in samples.

The average bacterial population calculated by method I for 14 samples collected at all five stations was 5.43 ± 0.37 with a variation coefficient of 25.2%. The bacterial population in the lake water was 6.10 ± 0.71 (as calculated for six samples taken at sts. 107, 113, and 114); and the average bacterial population in the water of the Komissarovka River and the First Erik River mouth was 4.93 ± 0.28 (as calculated for eight water samples taken at sts. 68 and 72).

The generation time varied from 23 to 89 h, averaging 37 ± 5 h. The minimum average generation time (31 ± 4 h) was observed in the coastal lake water, while the highest value of this parameter (42 ± 16 h) was observed in the First Erik River mouth, where the loess

Table 2. Generation time (g, h), daily bacterioplankton production (P, mg/(l day)), the coefficient of energy metabolism (K_2), and the daily productivity of bacterioplankton (P/B)

Date	Station location	g	P	K_2	P/B
Sept. 6	Coastal zone	30	0.11	0.49	0.56
Sept. 8	The same	42	0.09	0.53	0.39
Sept. 10	"	23	0.14	0.79	0.71
Sept. 12	"	28	0.15	0.70	0.58
Sept. 6	Komissarovka River	25	0.16	0.56	0.66
Sept. 8	The same	39	0.09	0.46	0.43
Sept. 10	"	41	0.07	0.55	0.41
Sept. 12	"	53	0.06	0.51	0.31
Sept. 7	First Erik River mouth	31	0.14	0.47	0.52
Sept. 9	The same	23	0.15	0.63	0.71
Sept. 11	"	89	0.04	0.31	0.19
Sept. 13	"	26	0.11	0.60	0.65



Coefficients K_2 versus BOD values in Lake Khanka in 1997.

density was lower than in the lake [3, 4]. Daily bacterial production varied from 0.04 to 0.16 mg C/(l day), averaging 0.11 ± 0.01 mg C/(l day). In the coastal water, this parameter comprised 0.12 ± 0.01 mg C/(l day). In the water of the Komissarovka River and the First Erik River mouth, daily bacterial production was slightly less (0.10 ± 0.04 mg C/(l day)). The specific bacterioplankton production (*P/B*) varied from 0.19 to 0.71 day^{-1}, averaging 0.51 ± 0.03 day^{-1} (Table 2).

An analysis of the distribution of dissolved organic matter (DOM) in the lake the water showed that, depending on the loess content of the water (2–154 mg/l) and the size of the loess particles, the adsorbed organic matter comprised from 60 to 90% of the total amount of DOM (1–20 mg/l). It was found that the adsorption of DOM on loess particles leads to the formation of the so-called organomineral complex (OMC), which represents a mineral loess particle inside an envelope made up of adsorbed organic matter and called chorion [3]. Bacterial cells tend to attach to the OMC, so that as many as about 25% of the bacterial cells present in the lake water cannot pass through 40-μm-pore-size filters.

The presence of readily oxidizable organic matter in water can be judged from a parameter called biochemical oxygen demand (BOD). A comparison of BOD and K_2 values for the same water samples showed that there exists a positive correlation [1] between these parameters with the correlation coefficient $r = 0.61$ and $t_{st} = 1.93$ versus $t_{st} = 2.36$ (see figure). When the proportion of bacterial cells attached to the OMC increased threefold (from 5 to 15%), the decomposition of organic matter per one bacterial cell (including an unattached one) increased by more than a factor of 1.5, and that of BOD, by a factor of 2. High concentrations of OMC promoted the degradation of organic matter in the lake water, as evidenced by the high values of K_2 ranging from 0.31 to 0.79 with a mean value of 0.55 ± 0.03 (Table 2). The minimum value of this coefficient (0.31) was observed in the water of the First Erik River mouth, where the generation time of bacterioplankton was 89 h.

S.N. Winogradsky wrote, "The important principles that an ecologist must be guided by during the investigation of a natural process are the determination of the physicochemical conditions under which this process occurs and the microorganisms involved, the isolation of microorganisms essential to this process, and the study of their physiology" [9].

Microorganisms are mainly distinguished by the type of nutrition (photo-, chemo-, and heterotrophy). This can be taken as a principle for distinguishing the particular groups of bacterioplankton. The microbiological analysis of lacustrine bacterioplankton by conventional methods using standard nutrient media showed that the content of heterotrophic bacteria in the water of the coastal zone and First Erik River mouth varied from 17 to 62%, whereas this value varied from 0.1 to 2.7% in the lake water.

The water of Lake Khanka was found to contain large amounts of terrigenous aleurites [4]. This suggests the presence in the lake of chemoorganoheterotrophic bacteria capable of decomposing poorly soluble calcium triphosphate into soluble phosphates and of poorly soluble potassium aluminosilicates into the soluble salts of silicon, potassium, and aluminum.

Table 3. Physiological groups of microorganisms detected in water samples taken at different stations

Selective medium*	Bacterial group	Station						
		113	72	68	68 (mud)	107	107 (mud)	114
1	Nitrogen fixers	+	+	+	+	+	+	+
2	Ammonia oxidizers	+	+	+	+(traces)	+	+	+
3	Nitrite oxidizers	+	+	+	+	+	+	+
4	Denitrifiers	+	+	+	+	+	+	+
5	Denitrifiers	+	+	+	+	+	–	+
6		+	+	+	–	+	+	+
7		+	+	+	+	+	+	+

* 1, Winogradsky medium; 2, liquid Winogradsky medium (detection of nitrites with Griess reagent); 3, liquid Winogradsky medium; 4, nutrient agar with 0.1% potassium nitrate and bromthymol blue; 5, Omelyanskii medium; 6, Pikovskaya medium; 7, Zak medium.

The results of differentiation of different physiological groups of microorganisms by plating water samples on standard selective media are summarized in Table 3. The water samples taken at all five stations were found to contain nitrogen fixers, ammonia and nitrite oxidizers, and denitrifying bacteria. In addition, the lake water contained some bacteria responsible for the supply of soluble compounds of potassium and inorganic phosphate, which are essential mineral nutrients for bacteria and algae. The presence of aluminum compounds in the water promotes the formation of suspensions adsorbing organic matter.

Silicate bacteria found in the lake water may be involved in the leaching of utilizable potassium and silicon compounds from the potassium aluminosilicates present in the lake aleurites. This supposition was confirmed in the laboratory experiments in which bacteria isolated from Lake Khanka water were cultivated in a liquid Zak medium [10] in the presence of aluminosilicate rock from the Sokalinskoe deposit in the Kemerov oblast. After cultivation, the culture liquid separated from the solid phase by centrifugation and filtration was found to contain the soluble compounds of potassium, silicon, and aluminum (Table 4).

Waters in the river mouths were characterized by the largest concentrations of heterotrophic bacteria and the highest values of DOM. The bacteria of groups 4, 5, 6, and 7 were able to utilize organic matter. The bacteria of groups 6 and 7 produced soluble compounds of potassium and inorganic phosphate, which are essential for bacterial and algal growth. Silicate bacteria (group 7) also produced soluble silicon compounds essential for the growth of diatoms. Aluminum compounds, even in insignificant amounts, promoted the formation of organic matter–adsorbing suspensions. The lake water also contained bacteria involved in the nitrogen cycle, i.e., nitrogen fixers, ammonia and nitrite oxidizers, and denitrifying bacteria.

Bacteria from Lake Khanka could grow in distilled water supplemented with a solid dispersed matter obtained from the lake water. In this case, bacterial growth was proportional to the content of this matter in the cultivation medium. For instance, after 17 days of cultivation, the maximum numbers of bacteria grown in media containing 0.06, 0.2, and 1 g matter/l comprised 0.8×10^7, 1.1×10^7, and 1.4×10^7 cells/ml, respectively. Bacterial growth was accompanied by an increase in the content of some chemical elements in the liquid phase, probably due to their leaching from the organomineral complex (Table 5).

The bacterioplankton of Lake Khanka noticeably responded to an increase in the concentration of organic matter in the nutrient medium. For instance, the addition of 1 g/l starch to the cultivation medium led to a decrease in the concentration of aluminum and to an increase in the concentration of vanadium in the medium (bacteria leached 1.13% of the vanadium present in the solid matter used as the growth substrate). In this case, the concentrations of boron, copper, and manganese in the medium increased by 90, 260, and 230%, respectively, as compared to the values shown in Table 5, column 3. These data suggest that starch added to the medium adheres to the organomineral complex [13] and intensifies the metabolism of bacteria attached to it.

Table 4. Bacterial leaching of aluminosilicate rock and accumulation of potassium, aluminum, and silicon in the incubation medium

Chemical element	Concentration (mg/l) in the medium after 27 days of incubation	
	without bacteria	with bacteria isolated from water taken at st. 113
Potassium	0.4	18.6
Aluminum	1.0	12.6
Silicon	2.9	25.1

Table 5. Bacterial leaching of solid matter from Lake Khanka and the accumulation of chemical elements in the incubation medium (% of their content in solid matter)

Chemical element	Control (without bacteria)	After 17-day incubation of bacteria with 1 g/l solid matter
Al	0.05	0.69
B	2.00	22.94
Ca	23.50	82.35
K	2.03	23.00
Si	0.69	5.00
Sn	7.40	25.00
Sr	0.00	2.45
Ti	0.00	0.12

When bacteria and the infusorian *Colpoda steinii* isolated from the lake were grown together in the same medium (1 g/l solid matter and 1 g/l starch in distilled water), the ash content of the liquid phase increased by 7.5 times. In this case, the concentrations of iron, silicon, manganese, and aluminum in the culture liquid increased 15-fold, 3-fold, 11-fold, and 2- fold, respectively. The concentrations of other chemical elements leached by the mixed culture of bacteria and infusorian made up 5.2% (strontium), 12.2% (nickel), 5.2% (lead), 90.0% (boron), 86.9% (potassium), 92.9% (calcium), and 25.0% (magnesium) of their contents in the solid matter used as the growth substrate.

To conclude, the staining of water samples with fluorescamin before the filtration of bacteria is the most adequate method for the enumeration of bacterioplankton in the loess-containing Lake Khanka. The number of fluorescamin-stained bacteria detected on filters and

microscopic slides was 5.02 ± 0.22 and 4.21 ± 0.35 million cells/ml, respectively. The number of erythrosin-stained bacteria detected on filters and slides was 2.60 ± 0.62 and 4.12 ± 0.61 million cells/ml, respectively. The mean generation time of bacteria comprised 37 ± 18 h; daily bacterial production was 0.20 ± 0.02 mg C/(l day); the specific productivity of bacterioplankton (*P/B*) was 0.56 ± 0.01 day^{-1}; and the mean K_2 value was 0.55 ± 0.02.

The data presented in this paper suggest that the chemoorganoheterotrophic bacteria of Lake Khanka are an important component (together with heliotrophs) in the primary stage of the food chain in this lake, whose activity leads to the destruction of the mineral nucleus of the OMC. The increase in the loess content of the lake water stimulated bacterial growth and intensified the cycles of potassium, silicon, and other biogenic elements, thus providing additional mineral nutrition to lacustrine hydrobionts. Starch added to the suspension of mineral particles adhered to the particle–water interface [13] and activated the metabolism of bacteria attached to this interface, thus promoting the bacterial degradation of the mineral nucleus of the OMC.

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