Evaluation of results of changes in bacterial strains in ecosystem of lake Baikal

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Abstract. In the paper, we evaluated the results of changes in the microbial community using molecular research methods. The authors analyzed strains of microorganisms isolated from different parts of the ecosystem of Lake Baikal: pelagial, littoral and benthic sediments. While analyzing we identified the presence of restriction endonuclease (RE) enzymes. It was established that RE were detected in bacteria isolated from samples taken in places of anthropogenic influence (Listvyanka, Baikalsk). As a result, 28 types of different RE were identified. When reviewing the results obtained, it was established that the formation of these enzymes in bacteria is associated neither with the season of the year nor with the year of sampling. The condition for the synthesis of RE enzymes in microorganism strains is the appearance of allochthonous microflora in the lake ecosystem. These are the littoral parts of the lake, where anthropogenic influence is observed. As a result, it was established that the biosynthesis of RE enzymes occurred in bacterial strains under the influence of human activity. It is especially significant that anthropogenic changes can be identified in the early stages of impact on the ecosystem of Lake Baikal.

1. Introduction

In recent years, microbiological monitoring has been developed using molecular research methods \cite{1,2}. Under the influence of human activity, there are significant violations of the evolutionarily established aquatic biocenoses, both freshwater and marine \cite{3,4}. Since microorganisms are the main agents responsible for the modification, degradation or detoxification of pollutants, they play an important role in their natural attenuation. When allochthonous microflora (bacteria, viruses) enter ecosystems, RE enzymes are synthesized in microorganisms. These enzymes are widely used in solving applied tasks: biotechnology, bioengineering, molecular biology and ecology \cite{5,6}. In recent years, there have been studies concerning the RE expression in the formation of antibiotic resistance of microorganisms \cite{7}.

In our work, we evaluated the results of microbiological monitoring using molecular methods for studying the variability of microbial strains by the presence of restriction endonuclease (RE) enzymes in the ecosystem of Lake Baikal. During the research (1991-2013), more than 1350 strains of microorganisms were isolated and analyzed from different parts of the Baikal ecosystem: the pelagial...
and the littoral of the lake, as well as benthic sediments of deepwater drilling. The anthropogenic influence on Lake Baikal is increasing year by year, especially in the littoral zones of the southern tip of the lake. This is due to the intensive development of domestic and international tourism in this area, the construction of private hotels, cottages, shops, cafes, bathhouses, saunas, pools, all located along the shore of the lake. It is obvious that along with household wastewater, many different microorganisms enter the ecosystem of Lake Baikal. The survival of microorganisms in water reservoirs is proven by the results of studies that detected bacterial strains resistant to a wide range of antibiotics [8,9]. The clarification of the role of microorganisms entering ecosystems under anthropogenic influence, the evaluation and analysis of the results obtained is one of the most urgent problems in environmental studies during monitoring observations. There are a lot of publications in the scientific literature devoted to taxonomic characteristics, genomic and phylogenetic analysis, and methods of cultivating microorganisms isolated from Lake Baikal [10-14].

2. Materials and methods

Material sampling and processing were carried out according to generally accepted approaches and methods. Water samples were seeded by pour plate method. LB medium was used as a nutrient medium. The cultivation of bacteria was carried out at a temperature of 25 °C for 48 h, then screening was performed [15]. When analyzing the results obtained, the method of correlation analysis was applied using standard parametric and non-parametric criteria [16]. When analyzing the results obtained, it was revealed that during the studies conducted, for the first time, new producer strains of RE enzymes were isolated from the ecosystem of Lake Baikal and identified, found only in areas of anthropogenic influence [17,18]. A similar result was obtained when searching for RE in the deep rift freshwater lake of Nyasa [19].

In clean background areas of the ecosystem of Lake Baikal, pelagial, “Baikal-93” deepwater drilling sediments, bacteria strains with RE enzymes are not detected at all. The explanation for this phenomenon is that, in bacteria, the RE enzyme “destroying” any foreign DNA molecule that has entered the cell performs a protective function. It is obvious that the appearance of “extraneous” viruses or bacteria in the process of human activity leads to the fact that the autochthonous bacterial strains synthesize the RE enzyme which cuts the extraneous DNA molecule into specific sequences and the allochthonous microflora dies. Many of the identified RE enzymes are unique, therefore, are used in scientific and biotechnological developments. When evaluating RE detected at different sampling points, it was found that the range of occurring RE is very different depending on the place of their sampling. The number of the detected RE varies from 1 to 7 [20].

To identify the conditions for the synthesis of restriction (RE) enzymes in the nearshore part of the ecosystem of Lake Baikal in a specific sampling location, during cluster analysis, we identified 28 various types of RE. The frequency of occurrence of RE enzymes can be divided into several types: 1) rare restriction enzymes found only in the strain of bacteria isolated from only one sample. These include: Accl, Neol, SaII, Scall, HpaII, Fun4HI, HgiEII, NruI, HgiCI, AvaII, MwoI, CauII; 2) RE with a relatively small distribution, identified in 2-4 samples. These are: XhoI, Bpu1I, EcoRV, BaII, XhoII, EspI, DraII, EcoRII, San96I, BamHI; 3) common RE identified in 6-13 samples: HaeIII, ClaI, MboI, PstI. The most common RE, MboI, was found in 14 samples, which is 21% of the total number of the samples studied. The remaining RE ranged from 1.5% to 15%.

To identify the distribution of RE by month, we grouped the frequency of RE occurrence, all other conditions being equal. Figure 1 visualizes the distribution of the detected RE in different months and shows the results of the cluster analysis of the binary data array.

The number of restriction enzymes isolated from microorganism strains in different months varies from 3 to 16 types. From the total amount of data, we can note the 8th and 11th month of the year (August and November). At this time, the number of the isolated RE was maximal (11 and 16, respectively). The binary distance between the months was quite significant (minimum 0.6 or 60%), which indicates significant differences in the spectra of RE produced by the bacterial community.
In order to characterize the long-term dynamics, we grouped the frequency of RE occurrence by year. The results and visualization of the number of the detected RE characterizing different years of sampling are presented in Figure 2.

It was established that when analyzing the interannual difference (from 2001 to 2009), the number of the detected RE ranged from 3 to 12 types during the year. The most prominent is 2008, when only 3 types of RE were found. This is most likely due to the small number of observations (selected samples) conducted that year. The cluster distances (binary distances) on the dendrogram vary from 0.35 to 0.90 (35-90%). 2002 and 2009 turned out to be close in terms of the RE spectrum analyzed. Compared with the rest of the observations, 2005 was the most distant in terms of cluster distance. The spectrum of RE detected during this year to a lesser extent overlapped with the spectrum of RE synthesized by bacterial communities in other years.
3. Conclusion
In the paper, the results of changes in the microbial community were for the first time evaluated using molecular research methods. It was proposed to use restriction endonuclease enzymes as a marker of anthropogenic influence on the lake ecosystem. Thus, the complexity of interrelationships in microbial communities, their variability and unpredictability in ecosystems such as Baikal, and all possible human effects, determine the need for improved microbiological monitoring. Particular attention should be paid to the methods of processing the results obtained. The assessment of the anthropogenic impact on the ecosystem of Lake Baikal using statistical methods allows us to identify cause-effect relationships. It was established that the formation of RE in the isolated bacterial strains is associated neither with the season of the year nor with the year of sampling. Amounts of RE produced always have the same order of magnitude. Most RE occur 1-2 times, rarely 3 RE synthesized by bacterial strains are detected in one cluster. The dominant condition for the synthesis of RE enzymes in microbial strains is the appearance of allochthonous microflora in the Baikal ecosystem under anthropogenic impact. When evaluating the results, it can be argued that in the littoral part of the lake there is an influx of bacteria from household wastewater from bathhouses, saunas, pools, as well as washouts of the nearshore part of the lake after tourists visit the lake. In the strains of bacteria isolated from the pelagial of the lake and deepwater drilling sediments, where anthropogenic impact is minimal or absent, no RE enzymes were found.

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