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Diversity of viral photosystem-II psb A genes in the largest channel reservoir of Kazakhstan.

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ABSTRACT

Cyanobacteria of the genera Synechococcus and Prochlorococcus are an important contributors to photosynthetic productivity in the water ecosystems. The discovery of genes (psbA, psbD) that encode key photosystem II proteins (D1, D2) in the genomes of phages that infect these cyanobacteria suggests new models for the regulation, function and evolution of photosynthesis in the immense aqueous ecosystems. Potential recombination of phage and host genes requires studying diversity of these genes in phages of different ponds. Here, using metagenomic data the largest channel reservoir of Kazakhstan samples, we show that the different viral psbA genes are presented in the environment.

Keywords: bacteriophage, diversity, psb A, phylogeny, cyanobacteria.

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INTRODUCTION

Cyanobacteria of *Synechococcus* and *Prochlorococcus* genera are the dominant components of the prokaryotic pikophytoplankton and contribute significantly to the efficiency of global photosynthesis. As primary producers of the hydrosphere, cyanobacteria synthesize up to 89% of organic compounds in the ocean [1-4].

In turn, the quantitative and species composition of the populations of *Synechococcus* and *Prochlorococcus* cyanobacteria depends not only on the availability of nutrients and the photosynthetic activity of radiation, but on lytic processes caused by cyanophages [5].

Recent studies have shown a correlation between genetic diversity of marine phages and cyanobacteria of *Synechococcus* genus. It was found that changes in the number and variety of bacteriophages occur before these changes take place in the *Synechococcus* community, therefore, it can be concluded that phages can manage the dynamics of growth of the host cells [6].

In addition, cyanophages are of significant interest to researchers due to the unusual structure of the genome, which not only carries the genes capable of participating in some of anabolic pathways of blue-green algae (stress-sensitive genes encoding chaperones and genes responsible for bacterial motility and chemotaxis) and some key genes of photosynthesis II (psbA, psbD, hliP, и PSII) [7-13], but successfully uses them for reproduction. The study of the diversity bacteriophages genes of the photosynthetic system II is of a great importance for understanding the evolutionary processes of emergence and development of chlorophyll-synthesizing system and bacteria viruses as a unique group of organisms. [14].

In our research, we conducted the study of the genetic diversity of photosynthetic gene psbA of cyanophages in artificially created reservoir Kapchagai, one of the largest reservoirs of Ile-Balkhash region of Kazakhstan. Ile-Balkhash basin is one of the largest lake ecosystems of the planet and is a unique natural complex. . Landscape-ecological assessment of the Ile-Balkhash region is characterized by increasing mineralization and pollution of surface and ground water, and reduction of bio-productivity and cleaning functions Ile river delta, degradation of wetlands, a progressive process of anthropogenic desertification [15]. Such a situation in the future may lead to a number of negative consequences as a socioeconomic (the detriment of agriculture and fisheries, water pollution by industrial waste) and environmental type (climate aridity, misbalance of the natural water and nature) [16-17]. One of the largest water bodies of the Ile-Balkhash region is Kapchagai reservoir, created by the construction of hydroelectric power stations. To fill the reservoir, were used 2 annual flows of the Ile river, making it the largest artificial reservoir in Kazakhstan and at the same time irreparably disturbing the ecological balance of the region. Therefore, in order to prevent environmental deterioration it is necessary to conduct diverse ecological monitoring of the reservoir, including the study of cyanophages biodiversity and their hosts as an essential component of aquatic ecosystem.

MATERIALS AND METHODS

Water samples were collected in the summer in the coastal areas of Kapchagai at a depth of 5-10 m. The coordinates of the collection were 43°52'53,5" north latitude, 77°16'24,9" east longitude. The choice of points to collect virus-containing samples was due to the fact that in the place of water exit through hydro scheme the mixing process of river waters with the waters of the reservoir is completed, which can create the best conditions for exploring the diversity of phage community. Coordinates of collection points marked on the GPS and «Google map» maps [18].

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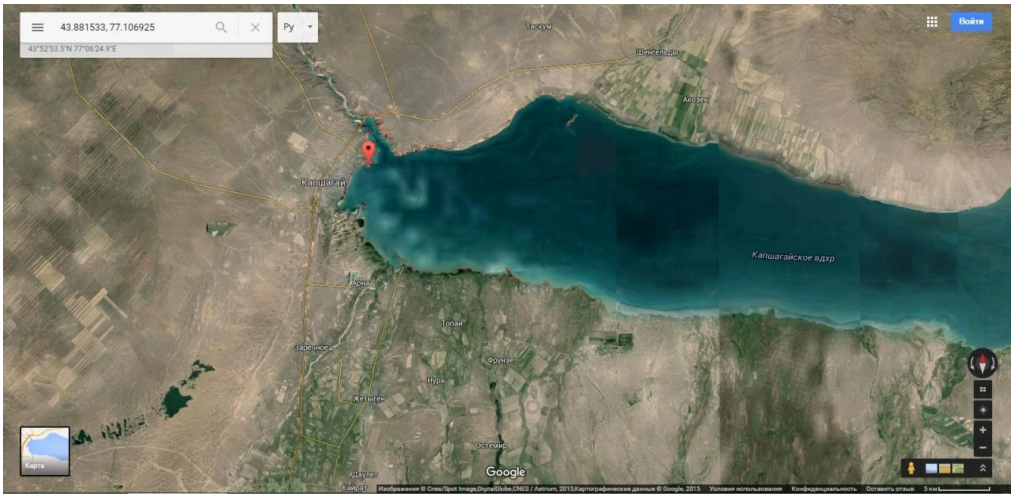

Figure 1 – Place of collection points of water sample

Sample volume of 1500 mL of water was sequentially filtered through polycarbonate filters (Millipore) with a pore diameter of 1.2; 0.45 and 0.22 microns to remove zooplankton, phytoplankton and bacterioplankton. Water was collected in sterile bottles and fixed with formaldehyde (final concentration 1%). Bacteriophages were precipitated by ultracentrifugation using corner - rotor «Beckman-L8-55», 100,000 g for 2 hours. The pellet was resuspended in a volume of 1.5 ml. DNA was isolated using Pure Link Viral DNA/ RNA Mini kit (Invitrogen) according to manufacturer's protocol. These samples yielded between 690 ng and 950 ng of DNA, using the Qubit dsDNA HS (High Sensitivity) Assay Kit (Invitrogen). Starting DNA amounts of 10 and 100 ng were used in Illumina sequencing library construction as described in the Genoscope protocol (Genoscope Illumina protocol) for sequencing on HiSeq.

Reeds were assembled in the standalone Edena software, freely available under the General Public License (GPLv3) at www.genomic.ch/edena.php, This tool is all publicly available, and currently often used to assemble short reads generated by next-generation sequencing platforms, such as Illumina Genome Analyzer (read length = 35–150 bp). In our studies with the purpose to find the greatest number of bacteriophages the required intersection of reads was set in 35 nucleotides. Simulated single-end and paired-end reads were generated from benchmark sequences with several variable parameters, including depth of coverage, base calling error rate and individual read length. Depth of coverage is the average number of reads by which any position of an assembly is independently determined [19]. As a source of benchmark sequences (viral genomes) we used a standalone version of the NCBI nucleotide database, comprising 6079 complete genomes of viruses.

Phylogenetic analysis Sequences were aligned and formatted using CLUSTAL W software BIOEDIT (v7.0.5) [20] and corrected manually with the help of the maximum-parsimony software (MEGA 6) [21]. Translated sequences were analyzed for the closest relatives by a BLAST search on the NCBI web site or ViroBlast (BLAST web server that allows users to search multiple sequence databases including public and local databases) [22]. The aligned sequences were compared with psb A fragments of known phages isolated from the *Synechococcus и Prochlorococcus.* Phylogenetic trees were reconstructed with the MEGA 6 or Lasergene sequence analysis software package (DNA Star software version 6.0, Madison, WI, USA).
The nucleotide sequences reported in this paper have been submitted to GenBank .

RESULTS

Psb A protein sequences

As a result of full-length sequencing of the virus containing DNA samples derived from Kapchagai reservoir and analyzis by standalone version blastn NCBI, 2.2.30 database and we have collected 736 sequences of *Synechococcus* and *Prochlorococcus* phages more than 150 nucleotides in length, of which 15 sequences of gene psbA were identified, forming the basis of the photosynthetic system II of blue-green algae. These 15 sequences had the length from 150 to 790 nucleotides, and were used for a comparative study of psbA gene diversity of phages in Kapchagay reservoir. It was found that identified gene sequences were clearly

divided into two clusters, each of which was subdivided into monophyletic group independent from each other (Figure 2).

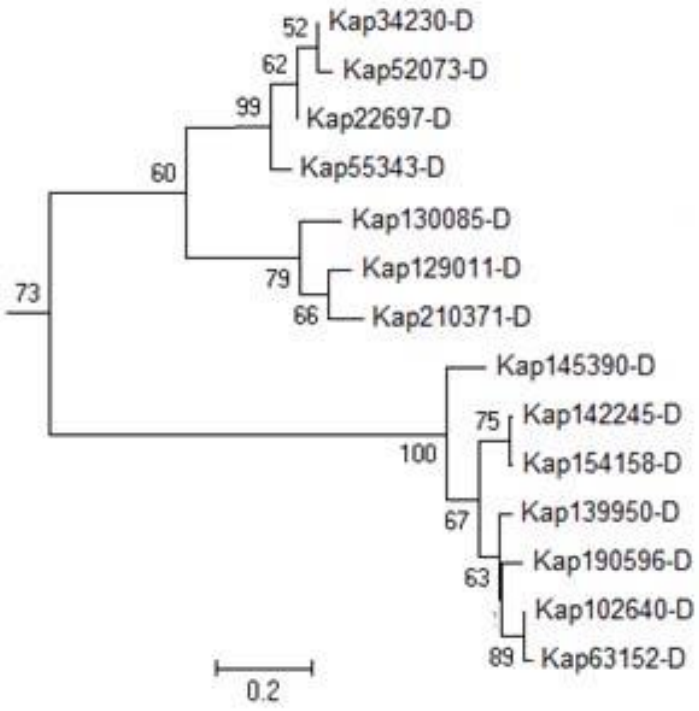

Figure 2. Evolutionary relationships of 14 sequences psb A genes of Kapchagai phages

The evolutionary history of psb A genes was then studied using the Neighbor-Joining method [23]. The optimal tree with the sum of branch length = 6.78940134 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [24]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [25] and are in the units of the number of base substitutions per site. The analysis involved 14 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 103 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [26].

Analysis of the similarities and differences between the psbA gene clones showed that monophyletic groups of each part are different from each other by not more than 10%, and the difference between the parts is more than 15%.

Phylogeny of D1 protein of photosystem II of phages isolated in Kapchagai

In further studies the comparison of sequences psb A protein of phage isolated in Kapchagai waterbody, with the same sequences identified in the lakes of Canada, the South China Sea, the subalpine lakes, rice fields, etc. was conducted (Figure 3). It is shown that the analyzed clones of protein psbA clearly divided into two groups: the first groups was the phages isolated mainly in waters with low temperature, the second is the other water bodies (rice fields, the sea, etc.). 13 investigated clones of psbA gene belongs to a group of phages isolated in high alpine lakes [8] or the Arctic Ocean [26], which may be due to runoff water from the mountain peaks of the Tien Shan in Kapchagai. The other clones correspond to the phages isolated in the sea or paddy fields.

Therefore, the study of the diversity of genes psbA phages isolated in large reservoirs in Kazakhstan showed that this waterbody absorbed water from different landscape zones and therefore is a unique place to study genetic diversity of cyanobacteria photosynthetic system II.

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The evolutionary history was inferred using the Neighbor-Joining method [23]. The optimal tree with the sum of branch length = 6.78940134 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [24]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [25] and are in the units of the number of base substitutions per site. The analysis involved 58 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 103 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [21].

DISCUSSION

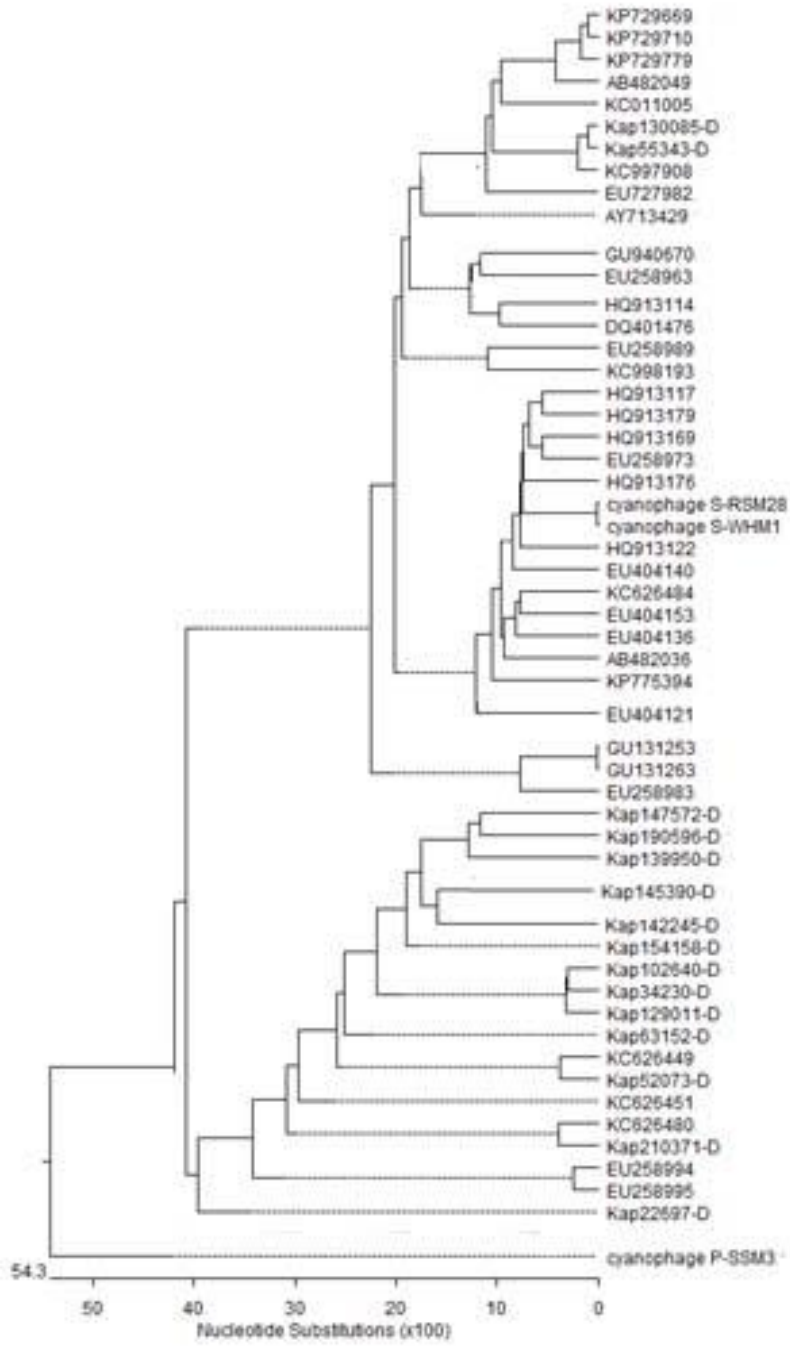

Figure 3. Evolutionary relationships of psb A genes different phages

A study of the diversity of D1 protein sequences of photosystem II of blue - green algae is of great importance. This is due a variety of reasons, the main ones being the understanding of the mechanism of evolutionary transformations and development of chlorophyll-synthesizing systems and reasons for the appearance of these genes in the viral genome that do not have their own protein-synthesizing apparatus. In addition, the parallel development of similar systems in microorganisms and viruses, a large number of recombinant transformations may give rise to understanding of the need for horizontal gene transfer. Such a study is not possible without a comparative study of gene sequences of viruses isolated from a single source. In our case, this is a unique hydraulic structure of Kapchagay waterbody, that contains in not only the flow of the Ili river, but also spring flood waters from the fields of agricultural, meltwater snowy mountains of Tien Shan, etc.

During metagenomic studies of viruses containing DNA samples were isolated at about 800 gene sequences of phages of blue-green algae, among which 15 gene fragments psb A were found, containing up to 50% of the nucleotides of the complete protein. It was shown that 15 clones gene fragments clearly divided into two groups. One of them corresponds to the sequences of phage isolated from the water with a low temperature, the other incorporates all other aquatic ecosystems.

An interesting fact is that, since Kapchagai accumulates runoff flowing on different landscape ecosystems, by studying a variety of phage community of the reservoir we could estimate biodiversity of gene clones psbA over vast geographical region that combines ecosystems of mountains, rice fields and sewage. It is also shown that in freshwater phages specific to South-East Asia and to Mediterranean, that can be explained by the presence of migratory waterfowl on the waterfront.

CONCLUSION

In conclusion, the present study demonstrated that psbA genes of *Synechococcus и Prochlorococcus* were highly diverse, suggesting a conceivable role of phages in the evolution of their hosts in the Kapchagay reservoir. In general, the psbA gene sequences from Kapchagai were closely related to cyanophages from marine, lakes and rice field environments. Further identification, isolation and molecular characterization of bacteriophages from various environments will allow us to obtain more accurate information about the phylogenetic relations within the phages of myoviridae genus and about the range of their hosts.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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