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# Biological and Genetic Characteristics of the Cholera Bacteriophage Rostov 7

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## Abstract

Currently a variety of genes encoding integrases, antibiotic resistance, and toxins had been identified which may be inserted into the phage genome. The study of isolated phages for the presence of these genes are highly relevant. The most informative method for identifying genetic determinants of pathogenicity factors and integrase is genome sequencing. The aim of our study was to study not only the biology of the cholera bacteriophage Vibrio phage Rostov 7, but also to conduct whole genome sequencing with a further assessment of the prospects for its practical use. The presence or absence of genetic determinants of resistance factors, toxins and integrase was checked with the help of the database created by us and the software developed, named "Phage Analyzer". The morphology of the cholera bacteriophage Rostov 7 corpuscles was determined by means of electron microscopy, and his specificity was confirmed. On the lawn of the indicator culture, the bacteriophage Rostov 7 forms transparent negative colonies with a diameter of 1.0 - 1.5 mm. Analysis of the nucleotide sequences revealed that its genome size is 45903 bp with the total number of ORF 35, genes characteristic of caudate phages were also found. The bacteriophage Rostov 7 was shown to have a separate position and to be unique. Two integrases were found in the genome structure (YP_009043902.1 and YP_009043892.1), therefore, the use of phage in prophylactic or therapeutic preparations is excluded, since it is moderate. Genetic determinants of resistance factors and toxins were not detected. At the moment the bacteriophage is actively used in the experimental work of the laboratory. Vibrio phage Rostov 7 can be successfully used to construct diagnostic products. The complete genomic sequence of Vibrio phage Rostov 7 is deposited in the international database NCBI GenBank under the accession number MK575466.1.

***Keywords:*** *Vibrio Phage Rostov 7; Myoviridae; Cholera; Sequencing; Bacteriophage*

## Introduction

The ongoing seventh cholera pandemic has attracted the attention of researchers to the problem of cholera vibrios. Bacteriophages are constant companions of cholera vibrios, and their presence in bacterial cells leads to the appearance of new properties [1]. Phages are conveniently used to solve such issues as the relationship of the virus with a bacterial cell [2], the effect of inactivating factors on phages, the variability of the properties of bacteria during the transfer of genetic information by phages, and the development of effective methods for the prevention and phagodiagnosis of pathogens.

Virulent forms of phages are the main element of the biological fight against bacterial infection. Their search and study of properties are of great interest [3]. Confirmation of virulence or moderation of the bacteriophage can be carried out during the identification of genes

encoding known integrase [4]. Phages are identified as virulent if they do not have an integrase gene. To date, many integrase, antibiotic resistance and toxin genes have been discovered that can be integrated into the phage genome. The study of the isolated phages for the presence of these genes is very relevant. The most informative method for identifying the genetic determinants of pathogenicity and integrase factors is genome-wide sequencing.

## Objective of the Study

To study the biological properties of the cholera bacteriophage Vibrio phage Rostov 7, as well as to conduct genome-wide sequencing with a further assessment of the prospects for its practical use.

## Materials and Methods

The bacteriophage Rostov 7, which we isolated earlier from the environment, was taken into the study. The source of phage isolation was water samples delivered for investigation to vibrio flora. Biological properties were studied by conventional methods [5]. Samples were examined with a JEM-1011 transmission electron microscope. Electron diffraction patterns were obtained using an Olimpus-SlS-Veleta CDC camera and iTEM-TEM imaging Platform software. Phage DNA was isolated in accordance with the previously described methods [6-8]. The amount and quality of the extracted DNA was controlled by electrophoresis in 0.8% agarose gel. The absence of bacterial chromosomes in the samples was confirmed by polymerase chain reaction (PCR) using gene-specific primers to determine hly and ctx + DNA fragments. The phage DNA solution was stored at a temperature of -20°C. The genomic sequence of bacteriophages was determined using high-throughput sequencing on the Miseq platform (Illumina). Assessment of the primary sequencing data was performed using the FastQC program [9]. For trimming and correction of reads, the algorithms Trimmomatic [10] and Lighter [11] were used. The assembly of genomes presented in the form of reads was carried out using the Spades program [12]. Comparison of the assembled genome with annotated sequences of known bacteriophages was performed using the BLASTN 2.2.29 algorithm (http://blast.ncbi.nlm.nih.gov). The presence or absence of genetic determinants of resistance factors, toxins, and integrase was checked using our database and the developed Phage Analyzer software, which allows us to quickly analyze the data of genome-wide bacteriophage sequencing.

## Results and Discussion

The bacteriophage Rostov 7 isolated from water bodies of the environment during monitoring of cholera on the lawn of an indicator culture forms transparent negative colonies with a diameter of 1.0 - 1.5 mm. Vibrio phage Rostov 7 is active against V. cholerae O1 serogroup of the Let or biovar. The specificity of the Rostov 7 phage with respect to the host was confirmed on a large set of representatives of closely related microorganisms of the families Vibrionaceae, Pseudomonadaceae, Enterobacteriaceae. The spectrum of lytic activity is 66.3%. Serological properties - VII serotype.

According to electron microscopy, the cholera bacteriophage Rostov 7 is classified according to the morphology of the corpuscles to the V morphogroup according to the classification of A. Tikhonenko. (1968) and type A of the family Myoviridae according to the classification of Ackermann H.W. (1987) (Figure 1).

Vibrio phage Rostov 7 has a shoot of complex structure, the cover of which is able to contract. Analysis of nucleotide sequences showed that the genome size of the bacteriophage Rostov 7 is 45903 bp with a total ORF of 35, genes characteristic of caudate phages were also found. By comparison with genes in the NCBI, 28 ORFs from the genus Vibrio and 7 ORFs from other genera with established functions were found.

Homologous sequences were discovered in the known bacterial genomes - hypothetical proteins of gamma-proteobacteria and prophage of Bacillus subtilis. Two integrases with a similarity of 96.6% (YP_009043902.1) and 94% (YP_009043892.1) were also found. No genetic determinants of resistance factors and toxins were found. After analyzing the data provided by the BLASTN system, only two cholera bacteriophages homologous to Rostov 7 were found - X29 (KJ572845.2) and phi2 (KJ545483.2) with 83% overlapping genes and

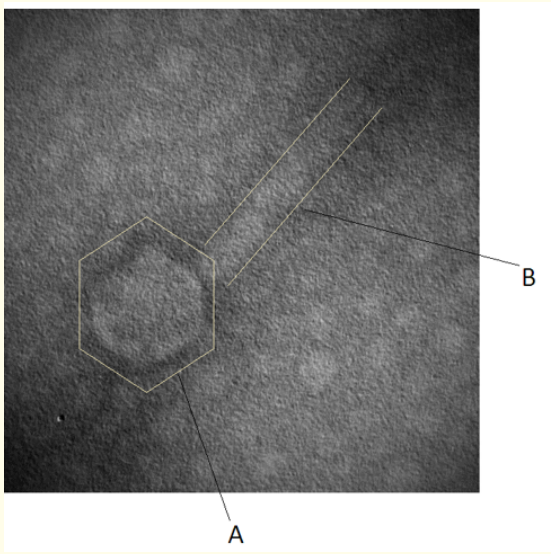


***Figure 1:*** *Morphology of the cholera bacteriophage Rostov 7 (magnification x150000), where A is a polyhedral head (453 × 510 Å) and B is a process (1023 Å).*

identical by 98.15% and 98%, respectively, from the Vibrio phage group (Figure 2). Integra genes were found in the sequences of the cholera phages found, just as in Rostov 7, which means they are moderate. Figure 2 shows that the percentage of coincidence of the Rostov 7 genome with the phages found in the BLASTN system is small. From the dendrogram in figure 3, it is clear that Vibrio phage Rostov 7 has a separate position, therefore it is considered unique.

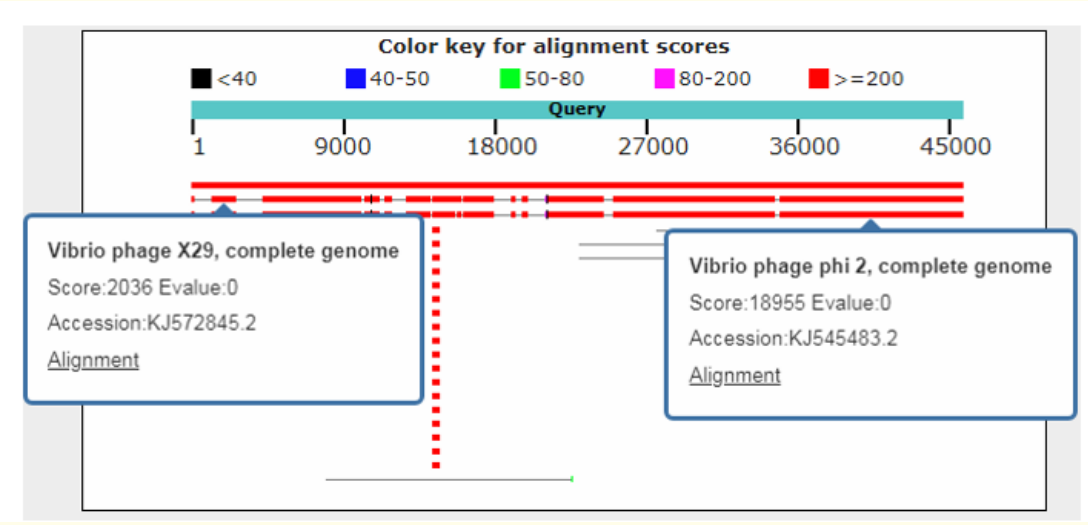


***Figure 2:*** *Alignment of the ORF system BLASTN.*

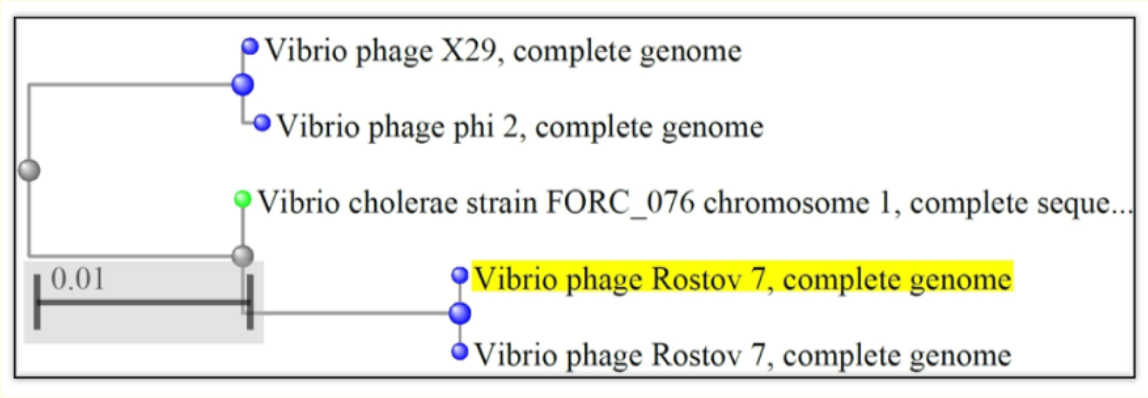


***Figure 3:*** *Dendrogram for Vibrio phage Rostov 7.*

## Conclusion

To characterize bacteriophages, the use of basic biological research methods is insufficient. Full genome sequencing provides more complete information about the structure and properties of phages and is also useful for obtaining reliable data for fine intraspecific differentiation of phages. As a result of our study, it was found that the Rostov 7 bacteriophage has a separate position and is unique. Two integrase were found in the structure of the genome; therefore, the use of phage in prophylactic or therapeutic preparations is excluded, since it is moderate. Currently, the bacteriophage is actively used in the experimental activities of the laboratory and for the construction of diagnostic drugs. The complete genomic sequence of Vibrio phage Rostov 7 is deposited in the international NCBI GenBank database under the number MK575466.1.

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