ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Study Of Morphology And Genome Of New Bacteriophages Hafnia Alvei.

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ABSTRACT

In the article the research results of 5 phages are presented, specific for bacteria Hafnia alvei. It was established that the phages refer to Caudovirales. By morphological characteristics hafnium phages H-1, H-2, H-3 и H-4 of УГСХА series refers to Myoviridae family – «with contractive tails of coat and central tube». Bacteriophage H-5 УГСХА is referred to Sifoviridae family– «bacteriophage with unconstructed tail». Genome of bacteriophage H-1 УГСХА was studied: 118 open reading frames were discovered which code proteins the size from 50 to 1400 of amino acid residues, the most presented sizes of proteins classes of 100-150 residues. Numerous homologies with proteins of various bacteriophages were discovered, which refer to Myoviridae (ncbi.nlm.nih.gov) family. On the basis of received data it can be concluded that bacteriophage H-1 УГСХА refers to Felix01 species (phylogenetic group)-of similar bacteriophages.

Keywords: bacteriophages, Hafnia alvei, morphology, genome, properties

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ISSN: 0975-8585

INTRODUCTION

Since 1940 bacteriophages have become one of the most popular objects in molecular biology. Research with the use of phage allowed to discover mechanisms of such fundamental biological processes as DNA replication, recombination, transcription and genetic regulation, Thanks to phage study possibility of development of many important methods and approaches of gene engineering were also discovered [1].

During last 3 decades one belief prevailed that phage study can't give the sciences anything crucially important. In Russia in the event and development of gene engineering cessation of interest to phage study took place, and only single scientific laboratories nowadays continue to concern themselves with bacteriophage tests. However , in spite of big cumulative scientific material about study of biological properties of bacteriophages, many questions demand additional search. So, for instance, at majority of deeply studied model colibacillosis phages of T-series, function of two thirds of their synthesized products were not established [2].

Besides there is no single scheme of taxonomy and morphological classification of these microorganisms, there is no standard sets of bacteriophages of many disease agents of animal and man, and also schemes and regulations of their appliance [3-4].

Interest to phages at native researches arose comparatively not long ago for a score of reasons and first of all becoming of multiply stable agents of different disease to antibiotics. Besides, nowadays as the result of molecular genetic research the connection of genome of pathogenicity of bacteria with prophage was discovered and possibility of genetic exchange not only between bacteria анв phages, but between phages and eukaryotes as distinguished [5-6]

Last years number of diseases of animals and men significantly increased. The diseases were caused by commensals or preceded with their participation [7-8]. One of such microorganisms is a representative of. Enterobacteriaceae family referred to Hafnia species with the only species of Hafnia alvei [9-12].

Unfortunately representatives of humane and veterinary medicine do not have in the inventory treatment and prophylactic and diagnostic bacteriophages Hafnia alvei.

Some strains of phage active to these microorganisms were detached from the objects of ambient by us. As they are new isolates, it was necessary to study main biological properties of these phages for the study of possibility to use them for applied aims.

For this reason the aim of our research was the electron microscopical study of our 5 detached strains of hafnium bacteriophages and study of genome of one and most perspective isolate.

MATERIALS AND METHODS

Electron microscopy of hafnium bacteriophage was conducted at microscope JEOL – 100CX.

For microscopy daily culture of bacteriophages was studied, preliminary filtering it through bacterial filters to clear phages from cellular debris. Further, on the sterile scaffolding a drop of phage was applied, then one after another 3 drops of double-distilled water and one drop of uranium acetate were put. As scaffolding special copper grids with the diameter of 3mm were used. They were covered with collodion support consisting of cellulose nitrate. The grid was made of 2% solution of cellulose nitrate in amino acetate. On the drop with phage copper grid was put and held for 5 minutes. Then we washed the grid in double-stilled water, step by step touching earlier prepared drops by it. After washing the grid was put on the drop with uranium acetate and held for 20 seconds. Then with the help of vacuum pump, all moistness was removed. Prepared products were watched under increasing in 80thousand times under strains of 80 kilo-volt.

The detachment of DNA of bacteriophage H-1 УГСХА was conducted according to the following method: one bacteriophage plague H-1 УГСХАwas added into the flash with 10 hour culture H. alvei 131. The flash with the culture and phage were put into thermostat for 18-20 hours. Detached substance was centrifuged during15 minutes at 15000 rotations for bacterial cells and lysate separation. Supernatant was

ISSN: 0975-8585

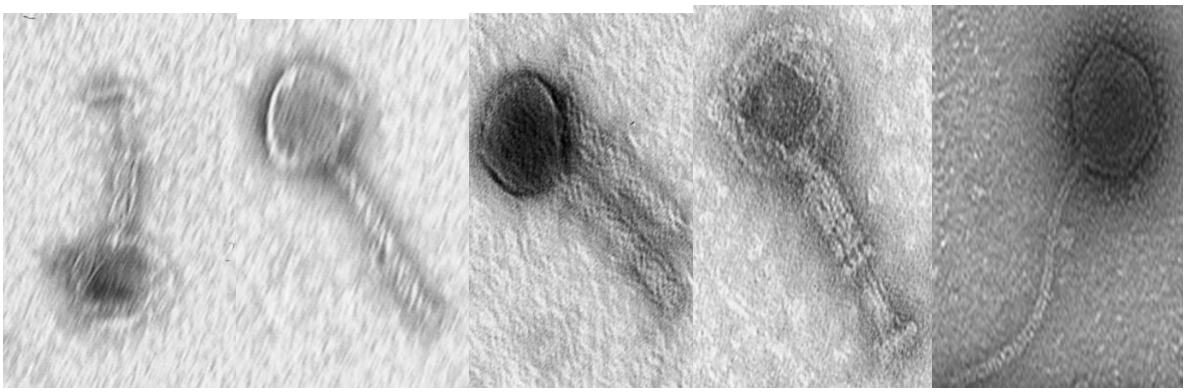
poured out into flashes of 150 cm^3 volume. 40 microliters of pancreatic DNase and RNase each were put into it for fermentation of residue of DNA and RNA of bacterial cell. Then the flash was put into thermostat for 40 minutes at a temperature of 37ºC, then it was centrifuged at 23500 r/min during an hour in order for phage particles to precipitate out, the supernatant was poured out and utilized.

In the flash with pallet 2 $см^3$ of physiological solution was added for dissolution of solid residue. Obtained solution was transferred into Eppendorf tubes in a volume of 0,85 $см^3$. in tubes with solution equal volumes of phenol were added, for proteolysis of phage and release of DNA, then they were centrifuged during 3 minutes at 13,5 thousand r/min. While centrifuging phenol and lysed protein precipitate, but DNA in the form of transparent jealous mass remains at the top.

Obtained DNA with the help of dropping tube was transferred into clear tubes, where phenol solution in equal volume was put, for the avoidance of hit into studied material of protein molecule. Then chlorophorm was added into tubes for settling of phenol. Earlier obtained substrate was poured into sterile tube with volume that is above the volume of Eppenorf tubes thrice. One tenth of volume of CH_3CONa (3-molar sodium acetate) was put for creation of salt background, under which DNA settles out. Tubes, where earlier CH_3CONa was put, 2,5 of 96% C_2H_5OH volume was added. Further we monitored clotting medusiform reaction, coming to the top of tube, that was taken and washed out of salts in 70% C_2H_5OH. Afetr washing it was dried in acetone. In coordination with acetone, medusiform reaction consolidated and became open for its physical separation. For sequencing a piece, the size of 1x1 mm was used, which was lysed in double-distilled water to the indiscrete mass. The left part of DNA was filled with 9% of spirit and kept at 3ºC.

RESULTS AND DISCUSSION

According to the results of electron microscopy of studied bacteriophages became obvious that presented hafnium phages are caudiferous phages relative to **Caudovirales (picture 1).**



Picture 1: Morphology of plague-forming unit of phages A) H -1 УГСХА, B) H – 2 УГСХА, C) H – 3 УГСХА, D) H – 4 УГСХА, E) H – 5 УГСХА

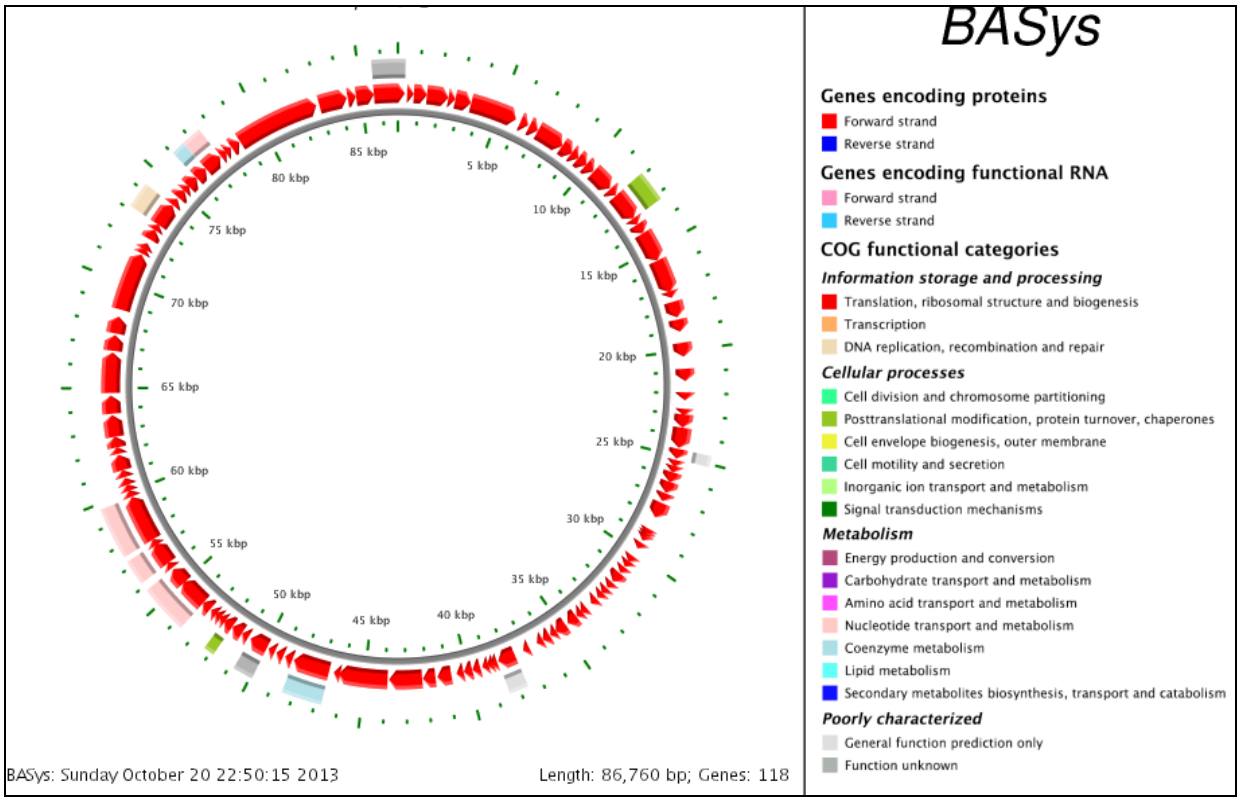
Caudiferous phages make the biggest and the most wide-spread group of bacterial viruses. According to literature data, in the world more than 4950 caudiferous phages were studied. They are probably the most ancient viruses, their birth can be dated by 3,5 billion years- by approximate age of the most ancient known microbial remains [13].

By morphological characteristics hafnium phages H-1, H-2, H-3 и H-4 we referred to Myoviridae family, with contratile tails, consisting of coat and central tube, phage H-5 we referred to Sifoviridae family– bacteriophage with unconstructed process. Capsids of mioviruses have a tendency to be bigger and contain more DNA than capsids of representatives of other families of caudiferous phages [14].

Such morphological groups were discovered by different researchers at other enterobacteria [15].

ISSN: 0975-8585

Whiley studying genome of bacteriophage H-1УГСХА, which was characterized earlier on the basis of study of its main biological properties as productive-perspective (of lytic activity, rage if lytic action, specificity) linear DNA was made(in picture 2 it is shown graphically hoop-type), the size of unique sequence of genome is 86760 bps. Content of GC pairs in DNA is distributed through homogeneously.



Picture 2: Distribution of GC pairs through genome of H-1 УГСХА phage

Automated annotation is done with the help of online service https://www.basys.ca/server4/basys/cache/4edbb80507c84b04d88ebe0142a0df0b/index.html. In genome 118 open reading frames were found (table and gene map) encoding proteins of 50 to 1400 of amino acids residues, more presented dimensional classes of proteins of 100 and 150 residues. Protein sequencing, encoded by phage, was compared with GenBank with the help of BLAST programm (ncbi.nlm.nih.gov). Numerous homologies with proteins of different bacteriophages, referring to Myoviridae family were discovered. On the basis of received data it can be concluded that bacteriophage H-1 УГСХА refers to Felix01 species (phylogenetic group) - of similar bacteriophages.

CONCLUSION

The research of 5 phages was conducted, specific for bacteria Hafnia alvei, detached by the authors independently. During the study of bacteriophage morphology by method of electron microscopy, it was established that the phages Hafnia alvei refer to Caudovirales. By morphological characteristics hafnium phages H-1, H-2, H-3 and H-4 series УГСХА are referred to Myoviridae family – «with contracted tails, consisting of coat and central tube». Bacteriophage H-5 УГСХА is referred to Sifoviridae family – «bacteriophage with unconstructed process».

Genome of bacteriophage H-1 УГСХА was studied, which was characterized earlier on the basis of study of its main biological properties as productive- perspective (lytic activity, range of lytic action, secificity). 118 open reading frames were found encoding proteins of 50 to 1400 of amino acids residues, more presented dimensional classes of proteins of 100 and 150 residues. Numerous homologies with proteins of different bacteriophages were discovered, related to Myoviridae family(ncbi.nlm.nih.gov). On the basis of received data it can be concluded that bacteriophage H-1 УГСХА refers to Felix01 species (phylogenetic group)-of similar

bacteriophages. Detached and studied perspective strains of bacteriophage H. alvei H-1 УГСХА refers to morphological family Myoviridae and Felix01 species (phylogenetic group)-of similar bacteriophage.

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