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Proteomic Analysis Of Bacteriophage Pr – 6 UGSHA.

Natalya A. Feoktistova[1], Dmitriy A. Vasilyev[1], Andrey V. Mastilenko[1], Ekaterina V. Suldina[1], Sergie N. Zolotukhin[1], Alexander L. Toigildin[1], Irina A. Toigildina[1], Alexander V. Dozorov[1], Vitality A. Isaichev[1], Alexander A. Nafeev[1], Igor L. Obuhov[2], Boris I. Shmorgun[3].

[1]Ulyanovsk State Agricultural University named after P.A. Stolypin, Ulyanovsk (Russian Federation).
[2]All-Russian Research Institute of Veterinary sanitary, Hygiene and Ecology, Moskau (Russian Federation).
[3]Federal State Budgetary Institution «The Russian State Center of Quality and Standardization Medicines for Animals and Feed» Moskau (Russian Federation).

ABSTRACT

In the article the results of study of proteomic analysis of bacteriophage of proteus Pr – 6 UGSHA (study of amino acid sequence of proteins, their qualitative and quantitative composition, isoelectric point of proteins, molecular number) that was detached and selected in 2017 from the objects of ambient in terms of specificity and lytic activity. In experiments recourses of system SnapGene Viewer v.4.1.7 and ExPasy (https://web.expasy.org) and method of vertical electrophoresis in PAGE were used. Analysis of profilogram was carried with the use of software GelAnalyzer 2010. As the result of undertaken studies data of poteomic analysis is compared on the basis of conducted sequence and electrophoresis in PAGE . It is established that qualitative composition of proteins of bacteriophage Pr – 6 UGSHA matches such at annotated analogues, has clear homologies of nucleotide and amino acid sets. During the analysis of proteome of bacteriophage Pr – 6 UGSHA and, consequently, data of sequencing its nucleonic acid 50 proteins with molecular numbers from 5,5 to 140 kDa. During separation of detached and concentrated proteins of phage in PAGE by vertical electrophoresis method for Proteusphage Pr – 6 UGSHA 3 proteins were determined (67 kDa, 77 kDa and 94 kDa). Obtained data about genome of bacteriophage Pr – 6 UGSHA, specific for bacteria *Proteus*, inducing zoogenous infections, draws us nearer to the creation of phage treatment medications of new- generation, adapted for parenteral administration, having established parameters of pharmacokinetics and complainting with modern standards of biological safety.

Keywords: *Proteus*, bacteriophage, proteomic analysis, sequencing, protein, molecular number

Corresponding author

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INTRODUCTION

Based on literature data it can be confirmed that in small intestine of young livestock animals and poultry in farms, unfortunate by intestinal diseases, bacteria of *Proteus* species are registered around 20-50 % cases [1-2].

Development of ecologically clear and effective therapeutic agents for diagnosis, treatment and prophylaxis of bacterial infections, induced by bacteria of *Proteus,* includes search and selection of specific bacteriophages, upon which new biopreparations can be constructed [3-6].

Interest to bacteriophage study is supported, besides fundamental aspects, by possibility of their appliance as medicine. In last 20 years quick growth of number and variety of strains of pathogenic microorganisms, stable to small molecule antibiotics, stimulated search of therapeutic alternatives and bacterial infections control. For maximum effective and scientifically grounded appliance of bacteriophages in medicine, veterinary science, agriculture and aquacultures their detailed study and systematization on genome level is necessary, and also high degree of purification of applied phage preparations. Analogues research in field of proteome study of bacteria and specific to them bacteriophages are shown in range of publications of foreign scientists and Russian scientists. [7-14]

Research aim is conducting of proteomic analysis of bacteriophage proteus *Pr* – 6 UGSHA (study of amino acid sequence of proteins, their qualitative and quantitative compositions, isoelectric point of proteins, molecular number).

MATERIALS AND METHODS

Research object is bacteriophage *Pr* – 6 UGSHA, detached in 2017 by a group of authors from the objects of ambient, having following characteristics- diameter of PFU- 0,5±0,1 mm, titer by Gratia - $1,3\pm0,2x10^{9}$ BFU/ml, titer by Appelmann – 10^{-8}, stable to influence of trichloromethane during 15 minute and specific for cultures *Proteus mirabilis* and *Proteus vulgaris*, detached from pathological material and objects of sanitary inspection of animal and poultry rooms from farming, unfortunate by intestinal diseases in 2016-2017 rr. [15-17].

For proteic analysis we used recourses of the system SnapGene Viewer v.4.1.7 and ExPasy (https://web.expasy.org) and analysis of bacteriophage *Pr* – 6 УГСХА, was carried, active in relation to bacteriophage *Proteus* and physical and chemical characteristics of each proteins in their composition were given

For analysis of protein profilogram of detached bacteriophage *Pr* – 6 УГСХА we used method of vertical electrophoresis in PAGE. Analysis of profilograms was carried with the use of software GelAnalyzer 2010.

As a first step it was necessary to obtain maximum possible bacteriophage weight for sufficient visual detection after electrophoresis. Bacterial weight was cultivated during 24 hours on culture fluid. Then detached bacteriophages were spiked in titer 10^{9} BFU/ml at a rate of 1,0 ml to 10 ml of bacterial culture according to studied species . It was cultivated during 48 hours at 37 °C under aerobic conditions and adequate moisture. After that part of aliquote of culture *Pr. vulgaris 28* [17] with bacteriophage *Pr* – 6 UGSHA was studied by agar-layer method by Gratia for confirmation of phage titers and a part was used for obtainment of bacteriophage proteins .

For detachment and concentration of bacteriophage proteins *Pr* – 6 UGSHA the culture liquid was centrifuged for 20 min at 3000r/min (Centrifuge type MPW-310, Poland). Supernatant liquid, containing bacteriophages, was carried into clean glass- tube at a rate of 5,0 ml and it was exposed to ultrasonic disintegration at the mode of 10 micron with triple approaches by 60 seconds. Then 5,0 ml of sole solution of ammonium sulphate was carried. All the manipulations were conducted in cold. Sole solution was incubated during 1 hour at 4-8 °C, and then proteins were precipitated at 10000 r/min during 30 minutes (Centrifuge type MPW-310, Poland). Supernatant liquid was removed under visual control of presence of sediment.

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Sediment was diffused in 100 mql of buffer for electrophoresis. Large conglomerates of insoluble fraction of proteins and detritus were sedimented at 3000 r/min during 1 minute (Centrifuge type MPW-310, Poland).

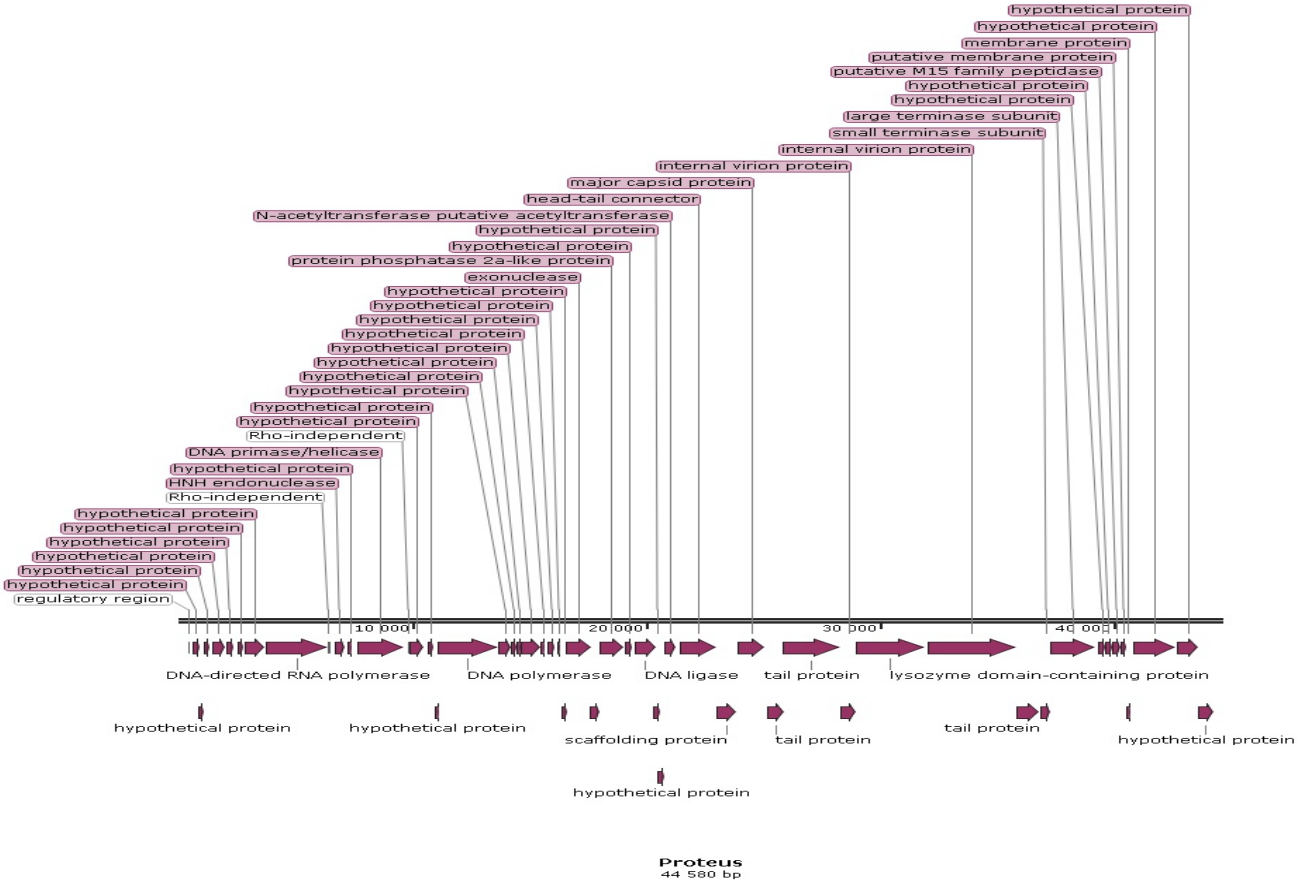
Detached and concentrated in such a way proteins were used for conducting of vertical electrophoresis in PAGE.

Mode of electrophoresis and concentration of PAGE: 200 V, 60 мА, 30 minutes, 4-20% PAGE, tris-glycine buffer with pH-8,6.

RESULTS AND DISCUSSION

As a result of conducted research we compared data of proteomic analysis on the basis of conducted sequence анв electrophoresis in PAGE. In picture 1 profilogram of proteomes of detached proteus bacteriophage is shown during their preparation in PAGE. For Proteusphage 3 proteins were discovered (67 kDa, 77 kDa and 94 kDa) (pic. 2-3).

During the analysis of proteome of bacteriophage *Proteus* according to data of sequencing of its nucleic acid 50 proteins with molecular number from 5,5 to 140 kDa were discovered. Qualitative proteomic composition of Proteusphage is shown in tables 1-2 and pictures 4-6.



Pic. 1 – Map of linear DNA of bacteriophage Proteusphage *Pr – 6* UGSHA with decoding of coding regions of genome

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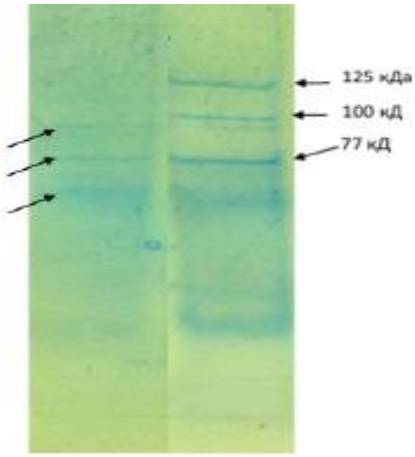
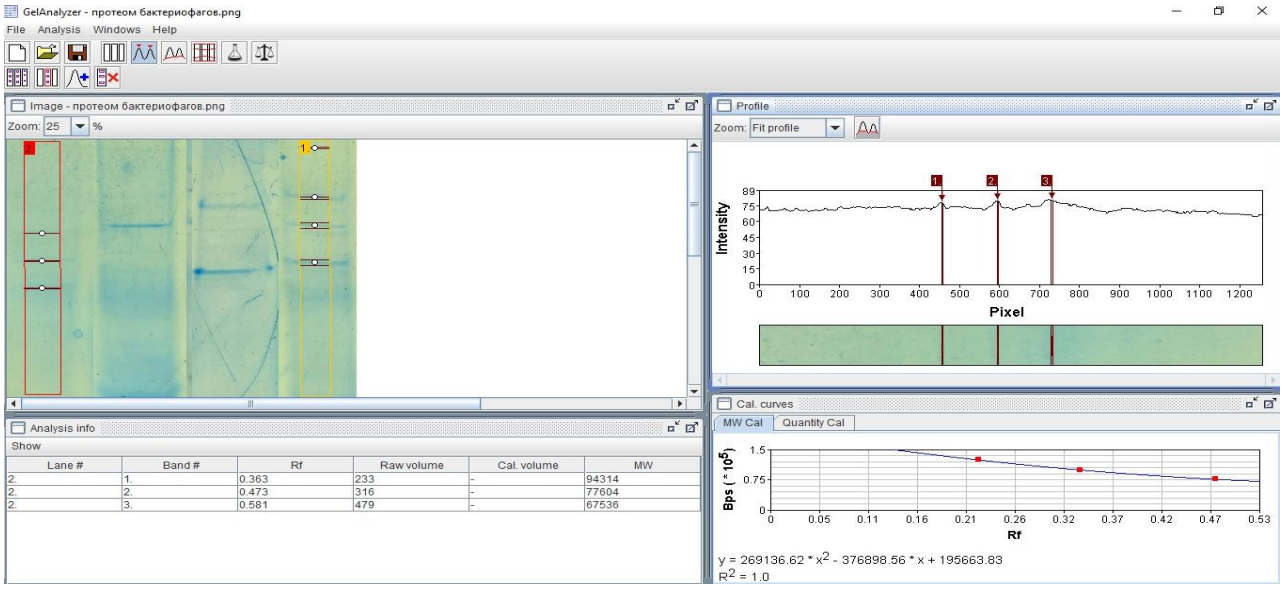
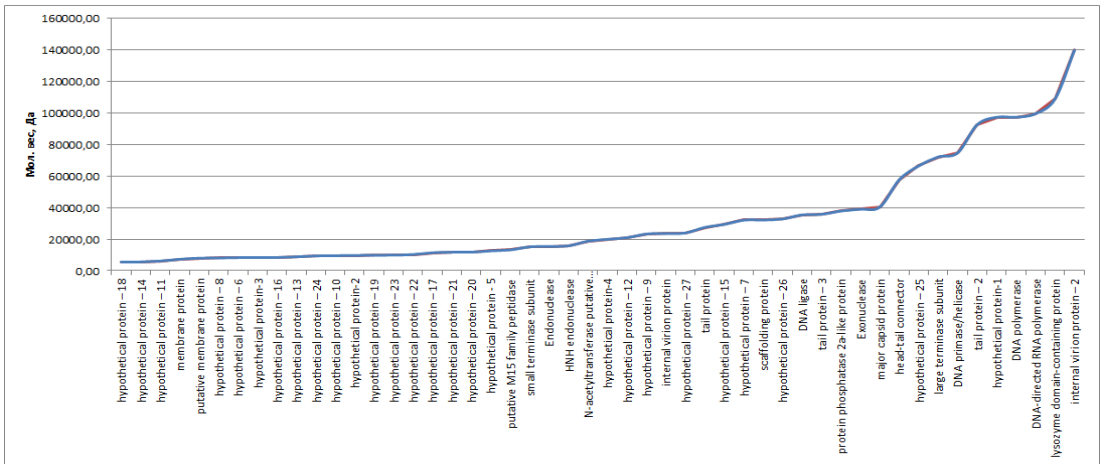


Pic. 2 – Profilogram of proteus of bacteriophage Proteusphage *Pr – 6* UGSHA and its comparison with marker



Pic. 3 – Analysis of proteomic bacteriophage Proteusphage *Pr – 6* UGSHA



Pic. 4 – Distribution graph of protein composition Proteusphage *Pr – 6* UGSHA by molecular number

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Table 1: Location of protein in genome Proteusphage

Sequence: Proteus.gb (Linear / 44 580 bp)
Features: 54 total

Feature	Location	Size	Type	
source	1 .. 44 580	44 580 bp	source	
✓ regulatory region	403 .. 422	20 bp	regulatory	
✓ hypothetical protein	607 .. 882	276 bp	CDS	hypothetical protein
✓ hypothetical protein	845 .. 1096	252 bp	CDS	hypothetical protein
✓ hypothetical protein	1096 .. 1305	210 bp	CDS	hypothetical protein
✓ hypothetical protein	1463 .. 1963	501 bp	CDS	hypothetical protein
✓ hypothetical protein	2028 .. 2357	330 bp	CDS	hypothetical protein
✓ hypothetical protein	2536 .. 2754	219 bp	CDS	hypothetical protein
✓ hypothetical protein	2825 .. 3670	846 bp	CDS	hypothetical protein
✓ DNA-directed RNA polymerase	3744 .. 6371	2628 bp	CDS	
✓ Rho-independent	6383 .. 6424	42 bp	regulatory	
✓ HNH endonuclease	6674 .. 7087	414 bp	CDS	HNH endonuclease
✓ hypothetical protein	7219 .. 7437	219 bp	CDS	hypothetical protein
✓ DNA primase/helicase	7647 .. 9635	1989 bp	CDS	
✓ Rho-independent	9815 .. 9858	44 bp	regulatory	
✓ hypothetical protein	9883 .. 10 494	612 bp	CDS	hypothetical protein
✓ hypothetical protein	10 636 .. 10 887	252 bp	CDS	hypothetical protein
✓ hypothetical protein	10 951 .. 11 115	165 bp	CDS	hypothetical protein
✓ DNA polymerase	11 099 .. 13 651	2553 bp	CDS	DNA polymerase
✓ hypothetical protein	13 690 .. 14 235	546 bp	CDS	hypothetical protein
✓ hypothetical protein	14 238 .. 14 468	231 bp	CDS	hypothetical protein
✓ hypothetical protein	14 487 .. 14 642	156 bp	CDS	hypothetical protein
✓ hypothetical protein	14 655 .. 15 461	807 bp	CDS	hypothetical protein
✓ hypothetical protein	15 465 .. 15 689	225 bp	CDS	hypothetical protein
✓ hypothetical protein	15 792 .. 16 115	324 bp	CDS	hypothetical protein
✓ hypothetical protein	16 187 .. 16 339	153 bp	CDS	hypothetical protein
✓ hypothetical protein	16 406 .. 16 651	246 bp	CDS	hypothetical protein
✓ exonuclease	16 597 .. 17 631	1035 bp	CDS	exonuclease
✓ endonuclease	17 616 .. 18 026	411 bp	CDS	endonuclease
✓ protein phosphatase 2a-like p...	18 019 .. 19 026	1008 bp	CDS	
✓ hypothetical protein	19 097 .. 19 402	306 bp	CDS	hypothetical protein
✓ DNA ligase	19 497 .. 20 438	942 bp	CDS	DNA ligase
✓ hypothetical protein	20 314 .. 20 625	312 bp	CDS	hypothetical protein
✓ hypothetical protein	20 498 .. 20 761	264 bp	CDS	hypothetical protein
✓ N-acetyltransferase putative...	20 761 .. 21 255	495 bp	CDS	
✓ head-tail connector	21 436 .. 22 986	1551 bp	CDS	head-tail connector
✓ scaffolding protein	22 986 .. 23 867	882 bp	CDS	scaffolding protein
✓ major capsid protein	23 941 .. 25 053	1113 bp	CDS	
✓ tail protein	25 182 .. 25 910	729 bp	CDS	tail protein
✓ tail protein	25 852 .. 28 317	2466 bp	CDS	tail protein
✓ internal virion protein	28 317 .. 28 994	678 bp	CDS	
✓ lysozyme domain-containing p...	29 003 .. 31 954	2952 bp	CDS	
✓ internal virion protein	32 022 .. 35 843	3822 bp	CDS	
✓ tail protein	35 843 .. 36 802	960 bp	CDS	tail protein
✓ small terminase subunit	36 870 .. 37 292	423 bp	CDS	
✓ large terminase subunit	37 292 .. 39 190	1899 bp	CDS	
✓ hypothetical protein	39 357 .. 39 632	276 bp	CDS	hypothetical protein
✓ hypothetical protein	39 644 .. 39 913	270 bp	CDS	hypothetical protein
✓ putative M15 family peptidase	39 923 .. 40 276	354 bp	CDS	
✓ putative membrane protein	40 303 .. 40 527	225 bp	CDS	
✓ membrane protein	40 520 .. 40 717	198 bp	CDS	membrane protein
✓ hypothetical protein	40 831 .. 42 675	1845 bp	CDS	hypothetical protein
✓ hypothetical protein	42 734 .. 43 606	873 bp	CDS	hypothetical protein
✓ hypothetical protein	43 606 .. 44 250	645 bp	CDS	hypothetical protein

Table 2: Proteomic composition of bacteriophage *Pr – 6* UGSHA

Item	molec. numb, Da	pI
DNA ligase	35410	6,57
DNA polymerase	97367	6,09
DNA primase/helicase	74770	5,92
DNA-directed RNA polymerase	99625	6,38
Endonuclease	15439	9,82
Exonuclease	39003	6,27
head-tail connector	57724	5,48
HNH endonuclease	15818	9,74
hypothetical protein – 1	97367	6,09

hypothetical protein – 10	9551	10,06
hypothetical protein – 11	6135	4,04
hypothetical protein – 12	20960	5,49
hypothetical protein – 13	8786	8,54
hypothetical protein – 14	5587	5,11
hypothetical protein – 15	29386	4,79
hypothetical protein – 16	8410	6,16
hypothetical protein – 17	11408	4,57
hypothetical protein – 18	5538	4,12
hypothetical protein – 19	9808	6,27
hypothetical protein – 2	9677	5,81
hypothetical protein – 20	11778	7,74
hypothetical protein – 21	11748	4,94
hypothetical protein – 22	10275	9,15
hypothetical protein – 23	9892	9,74
hypothetical protein – 24	9419	4,61
hypothetical protein – 25	66538	7,51
hypothetical protein – 26	32842	5,60
hypothetical protein – 27	23953	6,28
hypothetical protein – 3	8340	4,28
hypothetical protein – 4	19922	4,32
hypothetical protein – 5	12585	3,59
hypothetical protein – 6	8298	5,09
hypothetical protein – 7	32074	5,12
hypothetical protein – 8	8060	9,27
hypothetical protein – 9	23289	9,65
internal virion protein	23624	7,97
internal virion protein – 2	139867	6,49
large terminase subunit	72173	6,50
lysozyme domain-containing protein	108926	5,52
major capsid protein	40443	5,18
membrane protein	7245	5,11
N-acetyltransferase putative acetyltransferase	18741	6,20
protein phosphatase 2a-like protein	37886	7,04
putative M15 family peptidase	13215	9,39
putative membrane protein	7982	9,86
scaffolding protein	32160	4,17
small terminase subunit	15141	6,72
tail protein	27466	5,94
tail protein – 2	92577	5,48
tail protein – 3	35803	4,87

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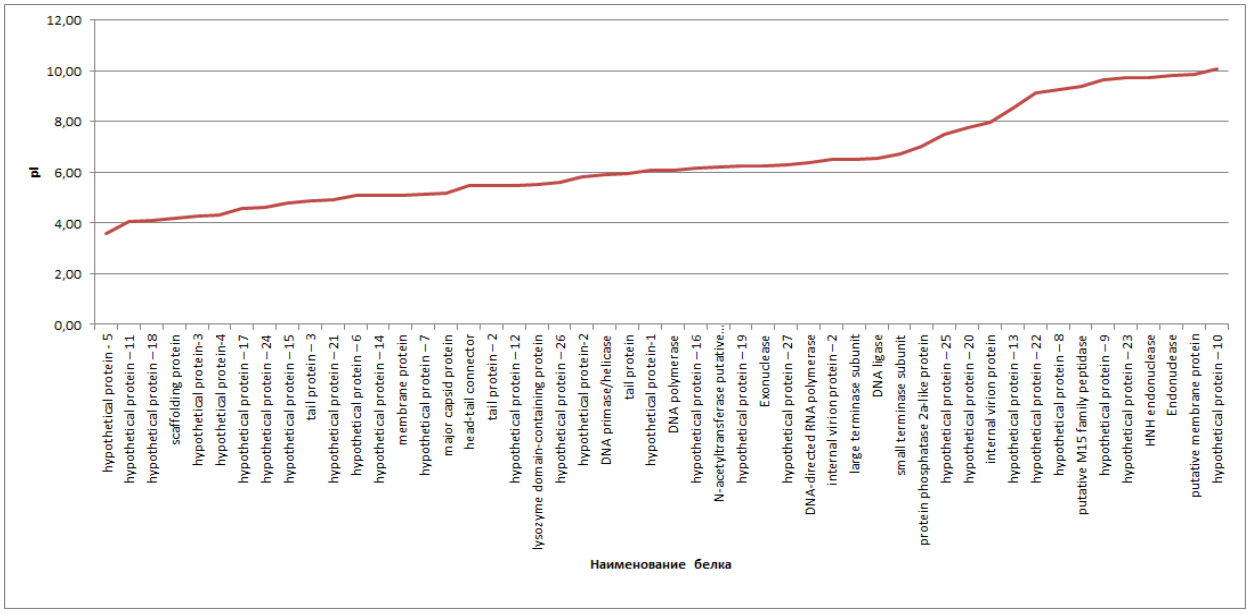
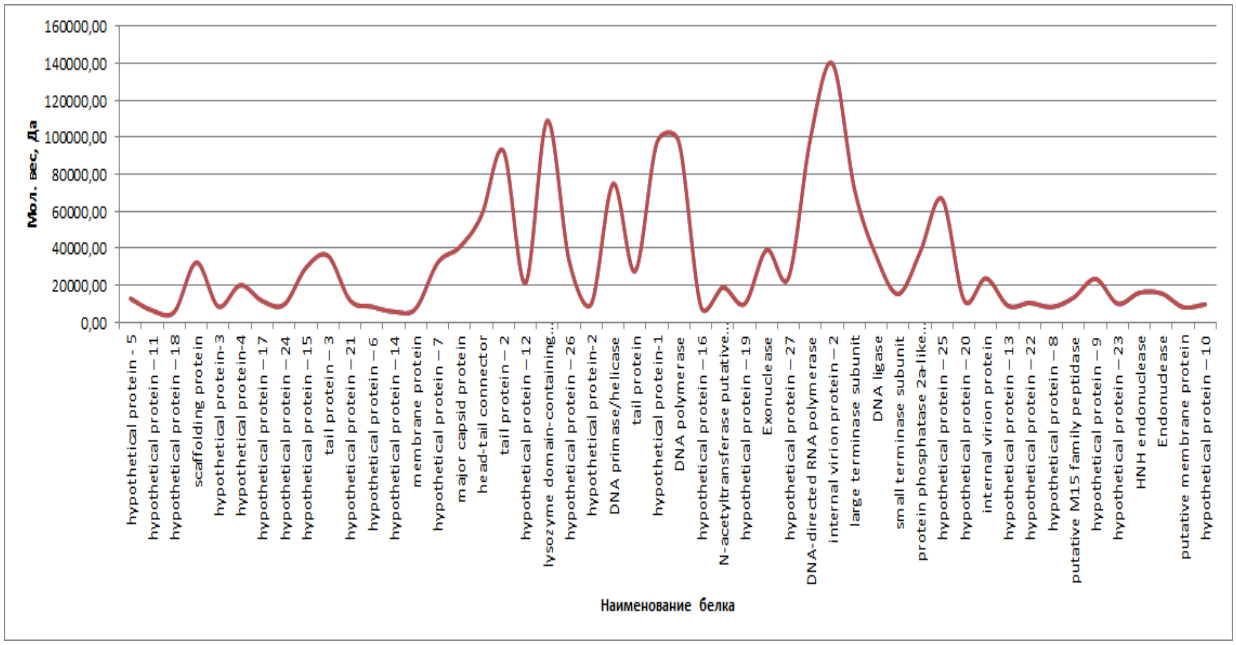


Pic. 5 – Distribution graph of protein composition of Proteusphage *Pr – 6* UGSHA by isoelectric point (pI)



Pic. 6 – Distribution graph of protein composition of Proteusphage *Pr – 6* UGSHA by molecular number depending on pI

CONCLUSIONS

As the result of conducted research sequencing data of genome of bacteriophage *Pr* – 6 UGSHA, obtained from objects of ambient and selected (by specific biological properties: lytic activity, specificity and spectrum of lytic action) and map of linear DNA was made with interpretation of coding region of genome. Data of nucleotide sequences of proteic bacteriophage, received at its sequencing, allowed us to carry comparative analysis of their genomes (table 1). We established that qualitative composition of proteins of bacteriophage *Pr* – 6 UGSHA corresponds such at annotated analogues , has clear homologies of nucleotide and amino acid sets. In the structure of proteins regularity was found, specific to bacteriophages- presence of structural and nonstructural components. Gene products were determined, having no clear determined functional characteristics-hypothetical proteins which have analogues in annotated genomes of bacteriophage, active towards bacteria of *Proteus* species. However study of biological properties of bacteriophages includes

also their proteic analysis. During the analysis of proteome of bacteria *Pr* – 6 UGSHA – data of sequencing of its nucleic acid, 50 proteins with molecular number from 5,5 to 140 kDa (pic. 4-6). During the separation of detached and concentrated proteins of phage in PAGE by method of vertical electrophoresis for Proteusphage 3 proteins were discovered. (67 kDa, 77 kDa and 94 kDa) (pic. 2-3). Received data about bacteriophage genome *Pr* – 6 UGSHA, specific for bacteria *Proteus* species, inducing zoonotic infection, moves us nearer to the creation of phage therapeutic medication of new generation, adaptated for parenteral appending, having established parameters of pharmacokinetics and conforming to up-to-date biological safety.

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