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Molecular-genetic Characteristics of Strains of Proteus Bacteriophages.

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ABSTRACT

In the article molecular genetic characteristic of sequeneed bacteriophages *Pr* – 6 УГСХА is presented. The map of linear DNA with decipher of coding sequence of genome is made. According to known analogues the expression products of their genes were identified. The scheme of molecular genetic indication (with the use of PCR) of autonomous genetic element (pathogenicity islands) in bacteriophages genomes was developed. Genomes which are active with relation to *Proteus ssp*. and supposed for appliance as therapeutic agents for treatment of enterobacterial infections, caused by above mentioned bacteria strains in veterinary medicine. According to the results of experimental research the indication of specific gene units *RelE of* culture *Proteus spp.* with developed system of oligonucleotide in genome of Proteus bacteriophage *Pr* – 6 УГСХА gene locus of pathogenicity wasn't determined. The data received allows to recommend bacteriophage *Pr* – 6 УГСХА, specific to bacteria *Proteus mirabilis and Proteus vulgaris species,* for engineering therapeutic biological product with the aim to prophylaxis and treatment of gastrointestinal diseases of agricultural rearers and poultry.

Keywords: *Proteus,* bacteriophages, genome, sequenation, primers, therapeutic agents.

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INTRODUCTION

The analysis of literature references indicates the fact that the unit of *Proteus* bacteria in small bowels of agricultural rearers and poultry in farmings, unfortunate in gastrointestinal diseases, can reach 50% [1-3]. Low percentage of lethality leads to high economic expenditures, as the decreasing of body weight of recovered animals and poultry and decreasing of immunological status in prospect leads to lack of growth per 1 food unit or 1 ruble of investments [4].

The search of non-polluting and effective therapeutic agents for treatment and prophylaxis of bacterial infections cannot exclude the appliance of bioproducts on the basis of specific bacteriophages [5-7]. In response to the features of its biology, bacteriophages can be powerful agents of lateral gene transfer from bacteria to bacteria. Bacteriophages, meant for the aim of phage therapy and phagoprophylaxis of infectious diseases, must be researched by methods of genomics for determination of their aptitude to transfer of bacteria genes [8]. The major routes of transfer and expression of «virulence genes» by bacteriophages are either lysogenic conversion (in case where genome of temperate phage contains unintended genes) or phage transduction. Genes, which can contain bacteriophages and the emerging of which in genomes of infected bacteria can cause unintended effect (for instance, high virulence). They can be divided into several groups according to their products: 1) genes of extracellular toxins; 2) genes, the products of which take part in affiction and oesizing of surfaces by bacteria; 3) gene enzymes, changing serotype of bacteria; 4) gene of proteins, helping to infection of bacteria into tissues. Naturally the presence of such genes in bacteriophages for the aims of therapy and prophylaxis of bacterial diseases is absolutely unacceptable. Apart the absence of virulent genes, phages must be absolutely lytic (virulent) and not cause the transduction of host DNA [9-12].

The research aim is conducting of molecular genetic research of *Proteus* bacteriophages for confirmation of originality, virulent nature and absence of pathogenicity locus

MATERIALS AND METHODS

Out of 94 tests from farmings, unfortunate in gastrointestinal diseases, bacteriophages, specific to *Proteus* bacteria, were detached. It was established that these bacteriophages were characterized by cytotoxic efficiency within the range from $4{,}2\pm0{,}2x10^6$ to $1{,}9\pm0{,}1x10^9$ BFU/ml (by A. Gratia method) and from 10^{-5} to 10^{-8} (by Appelman's method), were specific within species, they had cross lysis within *Proteus vulgaris* and *Proteus mirabilis* species. Cumulative percent of lysis of 8 bacteriophages for 42 cultures was 100 % [13-14].

The analysis of studied biological properties of bacteriophages *Proteus* allowed us to choose for further research , aimed to study of molecular genetic characteristic, including the determination of phage genome size, bacteriophages Pr – 6 УГСХА. The research was aimed to determine the percentage of its identity with taxonomically closest bacteriophages, to control the absence in DNA structure genes, encoding toxins, intergases, repressors of transcription and other undesirable locuses [15].

The whole genome sequencing of DNA of second generation bacteriophages was used. to obtain full based sequence (Ion Torrent, Thermo Fisher Scientific, the USA). Bacteriophage strains were sequences three times. The data of each round of sequencing were analysed by bioinformatics methods. Filtration of quality of reads allowed to collect genome of bacteriophage ith high validation.

In the research the libraries of data basis GeneBank (the USA), EMBL (European Molecular biological Library), DDBJ (DNA data bank of Japan) were used.

For PCR-sheets optimization, in reaction with *Proteus spp.* strains, electrophoretic method of detection of amplification products was used.

RESULTS AND DISCUSSION

In picture 1 detached genome was compared with known DNA of bacteriophages, deposited in GenBank NCBI, for determination of coding genome areas.

As the result of conducted research the map of linear DNA of detached and selected by us earlier bacteriophage *Pr* – 6 УГСХА was made. According to known analogues expression products of their genes were determined. Qualitative composition of proteins of bacteriophage matches such at annotated analogues, has distinct homologies of nucleotide and amino-acid sets. In protein structure regularity is observed, specific to given viral particles- presence of structural and nonstructural components. Also gene products were determined , not having distinct clear functional characteristics, so- termed hypothetical proteins, having analogues in annotated genomes of bacteriophages, active according to studied bacteria species. In table 1 bioinformatic analysis of conformality of known genes with data of given sequencing of bacteriophage is presented. On the basis of bioinformatic analysis of data of sequence the absence of pathogenicity locuses was proved. Otherwise the process of sequencing is rather expensive and complex, so in the given work we set ourselves the task to show the possibility of usage of PCR method to prove the absence of pathogenetic locuses in bacteriophage genome.

The next stage of research was system development of molecular-genetic indication(using PCR, polymerase chain reaction) of autonomous genetic element (pathogenicity islands) active according to *Proteus ssp.* and supposed for the appliance as therapeutic agents for treatment of enterobacterial infections, caused by above mentioned bacteria strains in veterinary medicine. On the first stage in the library of data basis GeneBank (the USA), EMBL (European Molecular biological Library), DDBJ (DNA data bank of Japan) the uniqueness of gene-candidate was determined and the fragment , coding *toxin RelE* was chosen.

Pic 1: The map of linear DNA of *Proteus* with a breakdown of coding genome areas.

Table 1: Bioinformatic analysis of compossibility of known genes with the data of sequencing of *Proteus* bacteriophage

After the analysis in libraries of data bases GeneBank (the USA), EMBL (European Molecular biological Library), DDBJ (DNA data bank of Japan)) of nucleic acid sequences of all above mentioned genes, they were scanned by the Blast system of data base GeneBank (the USA) on coincidence with sequence with acid sequences of DNA of known microorganisms. It was established that these genetic sequences are unique for *Proteus* phage toxin RelE and don't have concordance with other microorganisms.

After the choise of specific gene- candidate for molecular – genetic identification «pathogenic island», the agent of which can be bacteriophage, active towards *Proteus ssp*., the most conservative parts of chosen targets by their comparison in different strains of *Proteus* phage in data base GeneBank.

On these conserved parts by application Primer BLAST of this data base in on-line mode primers were placed, which satisfy some conditions, determine by us; primer length must be 18–24 of nucleotide pair, , temperature of primer melting must be 60–70 ˚C, size of flanked primer of genome part must be not less than 100 and not mor ethan 1000 base pair.

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Sequence: Proteus.gb (Linear / 44 580 bp)
Features: 54 visible, 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	845 .. 1096	252 bp	CDS	
✓	hypothetical protein	1096 .. 1305	210 bp	CDS	
✓	hypothetical protein	1463 .. 1963	501 bp	CDS	
✓	hypothetical protein	2028 .. 2357	330 bp	CDS	
✓	hypothetical protein	2536 .. 2754	219 bp	CDS	
✓	hypothetical protein	2825 .. 3670	846 bp	CDS	
✓	DNA-directed RNA polyme...	3744 .. 6371	2628 bp	CDS	
✓	Rho-independent	6383 .. 6424	42 bp	regulatory	
✓	HNH endonuclease	6674 .. 7087	414 bp	CDS	HNH endonucle...
✓	hypothetical protein	7219 .. 7437	219 bp	CDS	
✓	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	10 636 .. 10 887	252 bp	CDS	
✓	hypothetical protein	10 951 .. 11 115	165 bp	CDS	
✓	DNA polymerase	11 099 .. 13 651	2553 bp	CDS	DNA polymerase
✓	hypothetical protein	13 690 .. 14 235	546 bp	CDS	
✓	hypothetical protein	14 238 .. 14 468	231 bp	CDS	
✓	hypothetical protein	14 487 .. 14 642	156 bp	CDS	
✓	hypothetical protein	14 655 .. 15 461	807 bp	CDS	
✓	hypothetical protein	15 465 .. 15 689	225 bp	CDS	
✓	hypothetical protein	15 792 .. 16 115	324 bp	CDS	
✓	hypothetical protein	16 187 .. 16 339	153 bp	CDS	
✓	hypothetical protein	16 406 .. 16 651	246 bp	CDS	
✓	exonuclease	16 597 .. 17 631	1035 bp	CDS	exonuclease
✓	endonuclease	17 616 .. 18 026	411 bp	CDS	endonuclease
✓	protein phosphatase 2a-lik...	18 019 .. 19 026	1008 bp	CDS	
✓	hypothetical protein	19 097 .. 19 402	306 bp	CDS	
✓	DNA ligase	19 497 .. 20 438	942 bp	CDS	DNA ligase
✓	hypothetical protein	20 314 .. 20 625	312 bp	CDS	
✓	hypothetical protein	20 498 .. 20 761	264 bp	CDS	
✓	N-acetyltransferase putati...	20 761 .. 21 255	495 bp	CDS	
✓	head-tail connector	21 436 .. 22 986	1551 bp	CDS	
✓	scaffolding protein	22 986 .. 23 867	882 bp	CDS	
✓	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-containi...	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	39 644 .. 39 913	270 bp	CDS	
✓	putative M15 family peptid...	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 prot...
✓	hypothetical protein	40 831 .. 42 675	1845 bp	CDS	
✓	hypothetical protein	42 734 .. 43 606	873 bp	CDS	

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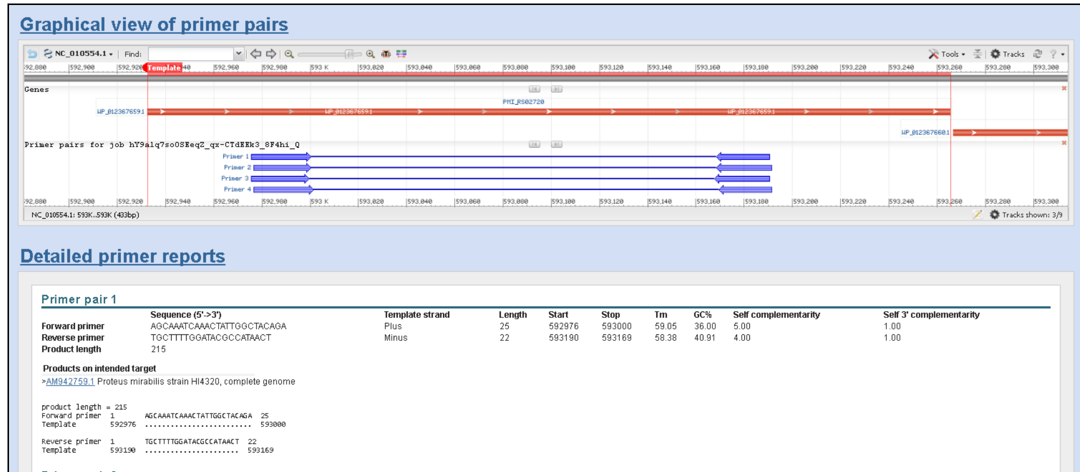
After determination of primers, they were alined by Gene Runner Version 3.05 programme, dimers were determined, during their non complementary fixation with themselves and in pairs. In finished variant of primers, theoretical specificity and fragments of amplifiable area are shown in picture 2 and in table 2.

For optimization of PCR-record, in reactions with *Proteus spp.*, detached from the sample of pathological material and poultry houses and objects of sanitary inspection of livestock and poultry houses from farmings, unfortunate in gastrointestinal diseases, electrophoretic method of detection of amplified products was used. The results of experiment research of indication of specific fragment of RelE culture *Proteus spp*. with developed systems of oligonucleotide are presented in picture 3.

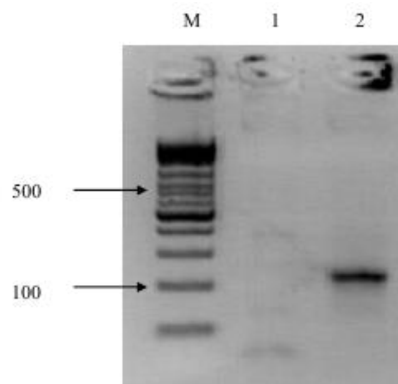


Pic 2: Variants of primer systems for amplification of gene *toxin RelE* phage genome, active towards *Proteus spp.*

Table 2: Characteristics of primers to regions of the gene *toxin RelE* pahge genome, active towards *Proteus spp*

Parameter	Characteristic
the gene toxin RelE	
Upstream primer (f) 5’-3’	AGCAAATCAAACTATTGGCTACAGA
Downstream primer (r) 5’-3’	TGCTTTTGGATACGCCATAACT
Predicted temperature of melting of upstream primer	60,0˚C
Predicted temperature of melting of downstream primer	59,9˚C
Theoretical specificity	*Proteus mirabilis, Proteus vulgaris RelE gene*
Length of amplified region (base pair)	215

As the result of conducted experiments in genome Pr – 6 УГСХА pathogene locuses were not found.



Pic 3: Indication of gene *RelE* fragments*:*

M – molecular weight marker, 1 – DNA of bacteriophage Pr – 6 УГСХА, specific towards *Proteus spp.*, 2 – positive control

As the result of conducted research sequenced data of genome bacteriophage *Pr* – 6 УГСХА were obtained, the map of linear DNA was made with decoding of regions of genome. In accordance with known analogues products of gene expression were determined. Qualitative composition of bacteriophage proteins matches the same in annotated analogue, it has distinct homologies of nucleotide and amino- acid set. In structure of proteins regularity is observed, typical for given viral particle- the presence of structural and nonstructural components. Also gene products were found, without distinct functional characteristics, so called hypothetical proteins, having analogues in annotated genomes of bacteriophages, active towards studied bacteria species. The system of molecular- genetic indication is developed (with the use of PCR) autonomous genetic elements (pathogenicity islands) in genoms of bacteriophages, active towards *Proteus ssp*. and supposed for appliance as therapeutic agent for treatment of enterobacterial infections, caused by above mentioned bacteria strains, in veterinary medicine. The uniqueness of gene-candidate is determined and fragment , encoding gene *toxin RelE* is chosen. Characteristic of primers to regions of the gene *toxin RelE Proteus spp.* phage genome, active towards*:* upstream primer (f) 5'-3' – AGCAAATCAAACTATTGGCTACAGA; Downstream primer (r) 5'-3' – TGCTTTTGGATACGCCATAACT; Predicted temperature of melting of upstream primer - 60,0 ˚C; Predicted temperature of melting of downstream primer - 59,9 ˚C; Theoretical specificity - *Proteus mirabilis, Proteus vulgaris RelE gene*; Length of amplified region (base pair) – 215.

CONCLUSIONS

As the result of experimental research of indication of specific fragment of gene *RelE* culture *Proteus spp.* with developed systems of oligonucleotide in genome of protein bacteriophage Pr – 6 УГСХА locuses of pathogenicity were not found. Data received allows to recommend bacteriophage Pr – 6 УГСХА, specific to bacteria *Proteus mirabilis* and *Proteus vulgaris* species, for construction of therapeutic biological preparation for prophylaxis and treatment of gastrointestinal diseases of agricultural rearers and poultry.

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