ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Molecular-genetic Characteristics of Bacteriophage *Bacillus cereus FBc – 28 UGSHA.*

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ABSTRACT

In this article molecular-genetic characteristics of sequenced bacteriophage *Bacillus cereus phage FBc – 28 UGSHA* is presented. The map of linear DNA with mapping of coding sequences a genome was constructed. According to the known analogues expression products of their genes were determined. It was established that the use of phenol-chlorophorm extraction leads to the best extraction of template DNA. In genome of bacteriophage *Bacillus cereus phage FBc – 28 UGSHA* the system of molecular-genetic indication (with the use of PCR) of autonomous genetic elements (pathogenic locuses) for specific cereus bacteriophages was developed- candidates for the new high effective phage biopreparation was developed with the aim to enforcement of mediated, allowing to eliminate (destroy) pathogenic microorganisms from edible raw material of animal origin and ready for the use of meat, fish, diary food products. Based on experimental research findings indication of specific fragment of gene HBL enterotoxin with developed systems of oligonucleotides in genomes of *Bacillus cereus phage FBc – 28 UGSHA* bacteriophage pathogenic locuses were not found.

Keywords: *Bacillus cereus,* bacteriophages, genome, sequencing, primers, products

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ISSN: 0975-8585

INTRODUCTION

According to Center for Disease control (CDC Foodborne Outbreak Online Database) in the USA every year more than 60.000disease cases are registered, diseased by bacteria *B. cereus*. Herewith it is noticed that some patients with vomit symptomatology during bacillary alimentary infection toxic syndrome is wrongly diagnosed , caused by *Staphylococcus aureus,* while false agent of diarrheal type of this toxicoinfection is considered *Clostridium perfringens* [1-3]. In this regard the control of *B. cereus* in some products is an obligate sanitary and epidemiological factor, approved in sanitary regulations and standarts 2.3.2.1078-01 [4].

It is known that diarrheal syndrome is usually connected with HBL complex of diarrheal enterotoxin, at least 14 other kinds of *Bacillu*s and *Paenibacillus*, species exist, among these are *Bacillus subtilis, Bacillus mycoides, Bacillus pumilus* and others, which cause, as it was established, food disease [5-6].

The process of implementation of mediated bioprocessing (treatment of food raw material and finished product, aiding increase of expiry date), allowing to eliminate (destroy) pathogenic microorganisms from edible raw material of animal origin and ready for the usage of meat, fish, diary products cannot exclude the appliance of bioproducts on the basis of specific bacteriophages [7]. Due to special properties of their biology bacteriophages can be powerful agents of horizontal gene transfer of genes from bacteria to bacteria/ Bacteriophages, supposed for processing food products, must be researched by genomics methods for determination of their aptitude to bacteria genes transfer [8-9].

The research aim is conducting molecular-genetic research of bacteriophage *Bacillus cereus phage FBc – 28 UGSHA* for confirmation of originality, virulent nature and absence of pathogenic locuses aimed to determine bacteriophage-candidate for carrying out mediate bioprocessing, allowing to eliminate (destroy) pathogenic microorganisms from edible raw material of animal origin and ready for the usage of meat, fish, diary products.

MATERIALS AND METHODS

Bacteriophage *Bacillus cereus phage FBc – 28 UGSHA* is obtained from sample of soil, it was characterized by cytotoxic efficiency 9,0±1,0x10^{11} BFU/ml by Gratia and 10^{-10} by Appelmann lysed 100 % out of 13 strains *Bacillus cereus*, detached by us from objects of veterinary sanitary service, (including edible raw material of animal origin and ready meat, fish, diary products) and identified with the use за some schemes [10-13].

For ontainment of full sized nucleotide sequence of bacteriophage genome, genome-wide sequencing of DNA bacteriophages of second generation was used. (Ion Torrent, Thermo Fisher Scientific, the USA). Bacteriophage *Bacillus cereus phage FBc – 28 UGSHA* was sequenced three times. The findings of each round of sequencing were analyzed by bioinformatics method. Filtering of read quality allowed to collect genome of bacteriophage with high confidence.

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

Spectrophotometric measurement of optical dense of the probe at 260 nm, 280 nm and 230 nm was conducted using spectrophotometer Nanodrop 2000/2000c (ThermoFisher).

For optimization of PCR – record, in reactions with strains *Bacillus cereus,* electrophoretic method of detection of food amplification was used.

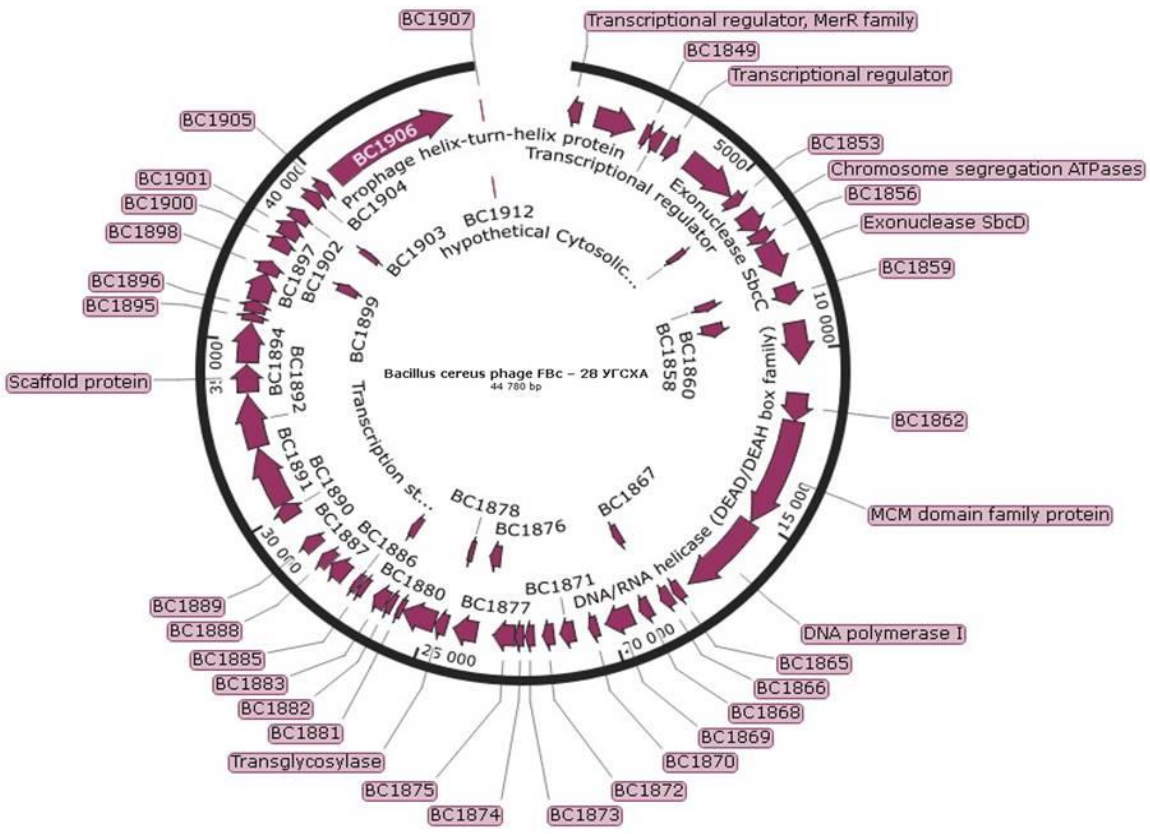
RESULTS AND DISCUSSION

The first stage of research of production-perspective strain *Bacillus cereus phage FBc – 28 UGSHA* bacteriophage was some research practices, oriented to determination of its molecular – genetic characteristic, including determination of phage genome size, percentage of its identity with taxonomically closest bacteriophage, control of absence in genetic makeup of DNA, intergases, transcription repressors and

other uncontrolled locuses. The research of given characteristics will allow us to confirm originality and virulent nature of bacteriophages.

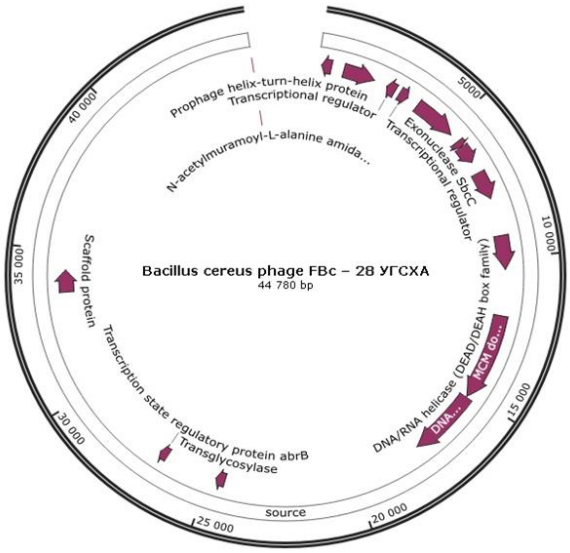
During sequencing database was obtained, it is shown lower for studied sequencing database was obtained, shown for studied sequencing database obtained, shown for lower for studied bacteriophage.

The results of each sequencing round were analyzed by bioinformatics methods. Filtering of read quality allowed to collect bacteriophage genome with high confidence. In picture 1-2 collected genome was compared with known DNA bacteriophage, deposited in GenBank NCBI for determination of coding genome areas.



Pic 1: The map of linear DNA of bacteriophage *Bacillus cereus phage FBc – 28 UGSHA* with decoding of coding genome areas

ISSN: 0975-8585



Pic 2: The map of linear DNA of bacteriophage *Bacillus cereus phage FBc – 28 UGSHA*. Gene mapping for which homologies are determined

As the result of conducted research maps of linear DNA detached and selected bacteriophage *Bacillus cereus phage FBc – 28 UGSHA* was made. According to known analogues products of expression of it genes were determined. Qualitative composition of bacteriophage proteins matches such at annotated analogues, it has clear homologies of nucleotide and amino-acid sets. In protein structure regularity is observed, specific for given viral particles-presence of structural анв non-structural components. Also products of genes were discovered, they didn't have distinct functional characteristics, known as hypothetic proteins, having analogues in annotated genomes of bacteriophages, active towards studied bacteria species.

One of the main criteria of biological properties of bacteriophage is the presence in structure of their genomes pathogenic islands. From the perspective of horizontal transfer of pathogenic genes at bacteria *Bacillus* species, important viral factor was enterotoxin, included in HBL-complex. Toxin structure is coded in HBL enterotoxin gene.

Within this work research in study of structure of given toxin, its most conservative areas were conducted. These areas will be used for development of detection system of given gene in bacteriophage genomes specific for «*Bacillus cereus*» group representatives, detached earlier by us.

On the first stage the analysis of base composition and enterotoxin gene alignment for discovery of conservative areas was conducted. Fragments of research are shown in picture 3.

On the basis of conservative areas of gene primers were chosen for detection of given gene by PCR method during bacteriophage screening. Its absence will show the perspective of appliance of given bacteriophages during treatment of edible raw material. Information of combination is shown in pictures 4-5.

ISSN: 0975-8585

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CLUSTAL W (1.81) multiple sequence alignment

5_Bacillus_cereus_14579                 ATGACAAAAAAACCTTATAAAGTAATGGCTCTATCAGCACTTATGGCAGTATTTGCAGCA
1_Bacillus_mycoides_ATCC_6462,          ATGACAAAAAAACCTTATAAAGTAATGGCTCTATCAGCACTTATGGCAGTATTTGCAGCA
2_Bacillus_mycoides_Gnyt1               ATGACAAAAAAACCTTATAAAGTAATGGCTCTATCAGCACTTATGGCAGTATTTGCAGCA
6_Bacillus_anthracis_SPV_842_15         ATGACAAAAAAACCTTATAAAGTAATGGCTCTTTCAGCACTTATGGCAGTATTTGCAGCA
3_Bacillus_cereus_FORC_047              ATGACAAAAAAACCTTATAAAGTAATGGCTCTATCAGCACTTATGGCAGTATTTGCAGCA
4_Bacillus_thuringiensis_Bt_1824        ATGACAAAAAAACCATATAAAGTAATGGCTCTATCAGCACTTATGGCAGTATTTGCAGCA
                                        ************ *************** *************************

5_Bacillus_cereus_14579                 GGAAATATTATGCCGGCTCATACGTATGCAGCTGAAAGTACAGTGAAACAAGCTCCAGTT
1_Bacillus_mycoides_ATCC_6462,          GGGAATATTATGCCAGCCCATACGTATGCTGCTGAAAGTACAGTGAAACAAGCTCCAGTT
2_Bacillus_mycoides_Gnyt1               GGGAATATTATGCCAGCCCATACGTATGCTGCTGAAAGTACAGTGAAACAAGCTCCAGTT
6_Bacillus_anthracis_SPV_842_15         GGGAATATTATGCCGGCCCATACGTATGCAGCTGAAAGTACTGTGAAACAAGCTCCAGTT
3_Bacillus_cereus_FORC_047              GGAAATATTATGCCGGCTCATACGTATGCAGCTGAAAGTACAGTGAAACAAGCTCCAGTT
4_Bacillus_thuringiensis_Bt_1824        GGAAACATTATGCCAGCTCATACGTATGCAGCTGAAAGCACAGTGAAACAAGCTCCAGTT
                                        ** ** ******** ** ********** ******** ** ******************

5_Bacillus_cereus_14579                 CATGCGGTAGCAAAAGCTTATAATGACTATGAAGAATACTCATTAGGACCAGAAGGCTTG
1_Bacillus_mycoides_ATCC_6462,          CATGCTGTAGCAAAAGCATATAATGATTATGACGAATATTCATTAGGACCAGAAGGCCTT
2_Bacillus_mycoides_Gnyt1               CATGCTGTAGCAAAAGCATATAATGATTATGACGAATATTCATTAGGACCAGAAGGCCTT
6_Bacillus_anthracis_SPV_842_15         CATGCGGTAGCAAAAGCTTATAATGACTATGAAGAATACTCATTAGGACCAGAAGGCTTA
3_Bacillus_cereus_FORC_047              CATGCGGTAGCAAAAGCTTATAATGACTATGAAGAATACTCATTAGGACCAGAAGGCTTG
4_Bacillus_thuringiensis_Bt_1824        CATGCGGTAGCAAAAGCTTATAATGACTATGAAGAATACTCATTAGGACCAGAAGGCTTA
                                        ***** ********** ******** ***** ***** ****************** *

5_Bacillus_cereus_14579                 AAAGATGCAATGGAAAGAACAGGTTCAAATGCTTTAGTAATGGATCTGTACGCTTTAACA
1_Bacillus_mycoides_ATCC_6462,          AAAGATGCGATGGAAAGAACAGGTTCTAACGCTTTAGTAATGGATCTGTACGCTTTAACA
2_Bacillus_mycoides_Gnyt1               AAAGATGCGATGGAAAGAACAGGTTCTAACGCTTTAGTAATGGATCTGTACGCTTTAACA
6_Bacillus_anthracis_SPV_842_15         AAAGATGCAATGGAAAGAACAGGTTCAAACGCTTTAGTAATGGATCTGTATGCTTTAACA
3_Bacillus_cereus_FORC_047              AAAGATGCAATGGAAAGAACAGGTTCAAATGCTTTAGTAATGGATCTGTACGCTTTAACA
4_Bacillus_thuringiensis_Bt_1824        AAAGATGCAATGGAAAGAACAGGTTCAAATGCTTTAGTAATGGATCTGTACGCTTTAACA
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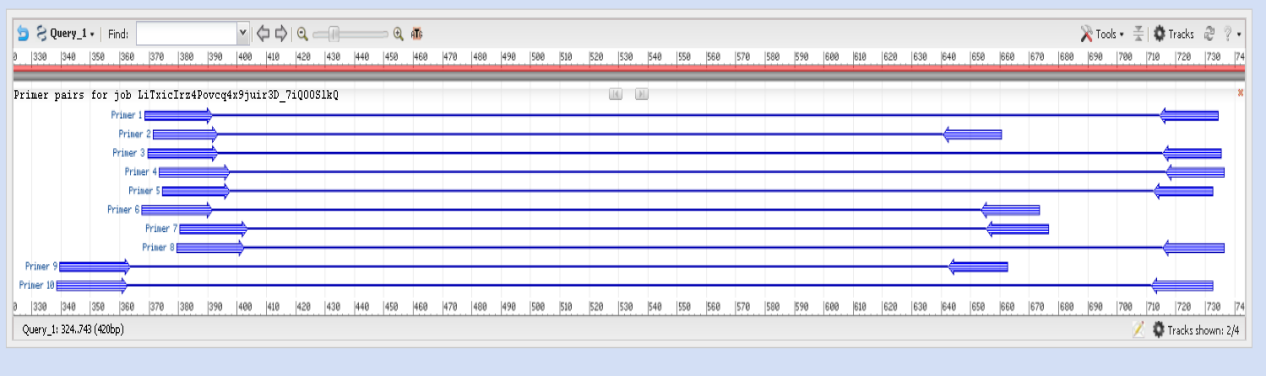
Pic 3: Enterotoxin gene alignment for discovery of conservative areas was conducted



Pic 4: The results of selection of primers to gene region *HBL* enterotoxin

ISSN: 0975-8585

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GAGATGCAAAAATTAATGCGGCG	Plus	23	369	391	60.06	43.48	6.00	3.00
Reverse primer	TGCGATTCCTAGCGGAGTTC	Minus	20	734	715	59.90	55.00	4.00	2.00
Product length	366								

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGCAAAAATTAATGCGGCGTA	Plus	22	372	393	58.22	36.36	6.00	3.00
Reverse primer	CTGTCGCAACAGATGAACCG	Minus	20	660	641	59.56	55.00	8.00	2.00
Product length	289								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGATGCAAAAATTAATGCGGCGTA	Plus	24	370	393	60.44	37.50	6.00	3.00
Reverse primer	ATGCGATTCCTAGCGGAGTT	Minus	20	735	716	59.25	50.00	4.00	0.00
Product length	366								

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCAAAAATTAATGCGGCGTATTGG	Plus	24	374	397	60.55	41.67	7.00	3.00
Reverse primer	AATGCGATTCCTAGCGGAGT	Minus	20	736	717	59.25	50.00	4.00	2.00
Product length	363								

Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CAAAAATTAATGCGGCGTATTGG	Plus	23	375	397	57.94	39.13	7.00	3.00
Reverse primer	CGATTCCTAGCGGAGTTCCT	Minus	20	732	713	58.97	55.00	4.00	2.00
Product length	358								

Primer pair 6

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGAGATGCAAAAATTAATGCGGCG	Plus	24	368	391	61.26	41.67	6.00	3.00
Reverse primer	ATTGGGCCTAAAGCTGTCGC	Minus	20	673	654	61.03	55.00	6.00	2.00
Product length	306								

Primer pair 7

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TTAATGCGGCGTATTGGTTAAAT	Plus	23	381	403	57.50	34.78	7.00	2.00
Reverse primer	GCAATTGGGCCTAAAGCTGTC	Minus	21	676	656	60.13	52.38	6.00	2.00
Product length	296								

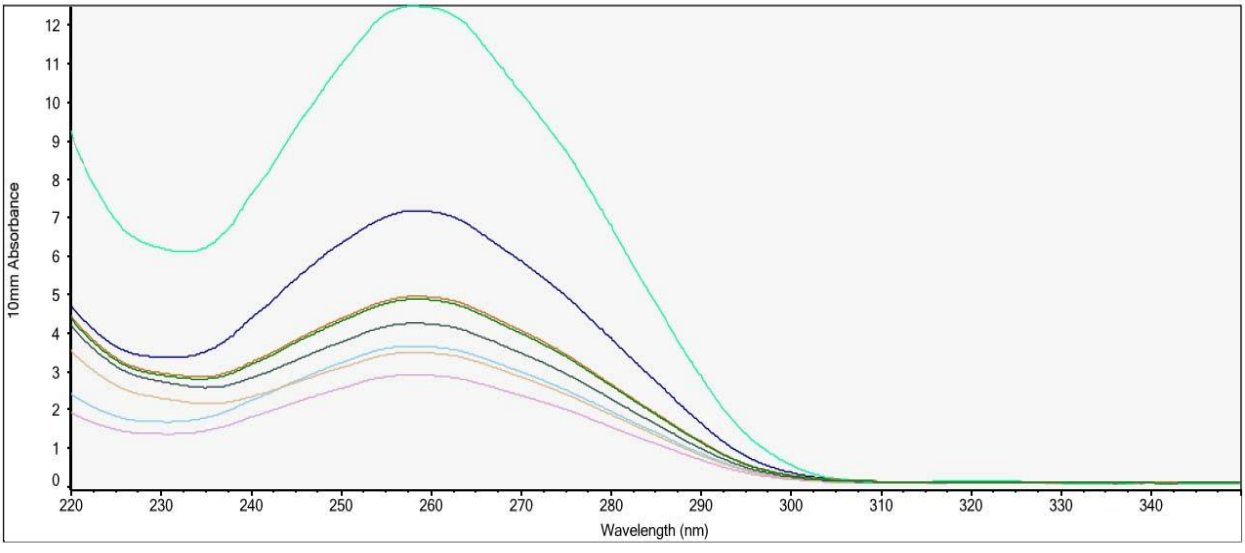
Pic 5: The results of selection of primers to gene region *HBL* enterotoxin

Ony of the important stages in practice of conducting molecular genetic experiments is extraction of nucleic acids. For this reason some different technologies of purification of nucleic acid from ferments, proteins, ions which can essentially complicate the processing of reaction, but in some cases it can ingibit the action of DNA- polymerase [14-15].

After the stage of extraction of nucleic acids spectrophotometric measurement of absorbency of each test at 260 nm 280 nm and 230 nm with the use of spectrophotometer Nanodrop 2000/2000 c (ThermoFisher). Herewith calculations A_{260}/A_{230} were used, allowing to abstract impurities not related to absorption of luminous power of nucleic acids. For determination of integrity of DNA calculations A_{260}/A_{280} were used, herewith the samples were considered with content of rather clear for appliance in PCR of DNA at a coefficient not less than 1,8. The results are shown in tables 1-2. Examples of diagram of absorption spectrum are shown in pictures 6-7.

Table 1: Calculation of coefficient of DNA cleanness with the use of sorption method with appliance of gaudinthiocyanate

Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
Bacteriophage *FBc – 28 UGSHA - Bacillus cereus*	242,7	ng/µl	4,854	0,511	1,91	0,85

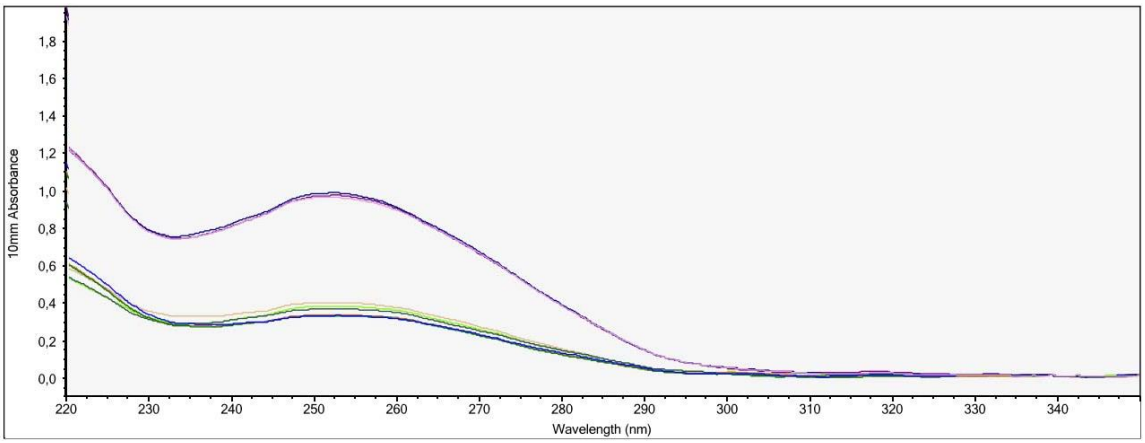


ISSN: 0975-8585

Pic 6: Diagram of spectrum of absorption of DNA preparations of bacteriophages detached with the use of sorbent method with gaudinthiocyanate

Table 2: Calculation of coefficient of DNA cleanness with the use of phenol-chlorophorm extraction method

Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
Bacteriophage *FBc – 28 UGSHA - Bacillus cereus*	48,7	ng/µl	0,973	2,594	1,87	1,69



Pic 7: Diagram of spectrum of absorption of DNA preparations of bacteriophages detached with the use of phenol-chlorophorm extraction method

After some conducted research and calculation of coefficient of cleanness of extracted nucleic acids it was inferred that the use of phenol-chlorophorm extraction leads to the best exit of template DNA, it was used in further research.

A pair of primers was determined, satisfying our optimal conditions GC not more than 60%, melting temperature ≈ 60°C, amplicon length from 200 to 400 bps., primer length from 18 to 24 bps (table 3).

Table 3: Characteristics of primers to gene HBL enterotoxin region

Parameter	Characteristic
gene HBL enterotoxin region	
Primer 1 (f) 5'-3'	GAGATGCAAAAATTAATGCGGCG
Primer 2 (r) 5'-3'	TGCGATTCCTAGCGGAGTTC
Predicted temperature of melting of upstream primer	60,0˚C
Predicted temperature of melting of downstream primer	59,9˚C
Amplicon size, bps	366

After selection of primer to gene HBL enterotoxin region one of the first tasks was determination of their capacity in tests, containing extracted DNA of above mentioned cultures. It was necessary to conduct a cycle of experiments with the use of polymerase chain reaction method with further detection by method oa horizontal electrophoresis in agarose gel.

The control of capacity and specificity of primers for genes GAGATGCAAAAATTAATGCGGCG and TGCGATTCCTAGCGGAGTTC regions, and also optimization of conducting mode of PCR (temperature control,

number of cycles, concentration of primers for reaction) were conducted on museum and field strains *Bacillus cereus,* and also on clinical samples.

Temperature of annealing of primers during conducting of experiments was selected by empiricism, based on their predicted temperature of melting. However it was necessary to choose such temperature as the annealing of primers for genes GAGATGCAAAAATTAATGCGGCG and TGCGATTCCTAGCGGAGTTC regions were proximal. It is necessary for making a universal protocol of conducting polymerase chain reaction during detection of DNA *Bacillus cereus,* as it will save not only time of research, but save operational life of amplifier, that is especially actual during conducting of mass analysis.

Range of temperatures, which we worked with, was rather high: from 50˚C to 75˚C. As the result of research of the first part of this stage of the project it was established that the annealing temperature 60 °C cam be used for primer system for identification of gene HBL enterotoxin. However, for primer systems for identification of gene HBL enterotoxin regulator on DNA matrix of bacteria group «*Bacillus cereus*»: *Bacillus cereus* demands optimization of amplification program and annealing temperature, as on electrophoregram there are unspecific amplicon band, having different lengths.

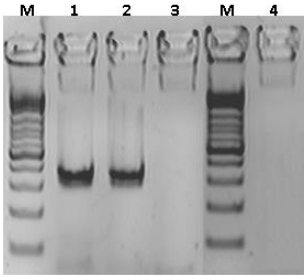
After some conducted research with diffeternt annealing temperatures, that 60°C for all systems of primers allows to get ampliconm specific for all researched targets. Also during the process of research optimal amplification program was determined allowing to get high specificity for primer systems for identification of regulatory gene HBL enterotoxin on DNA matrix of *Bacillus cereusv*b bacteria (table 4).

Table 4: Program for DNA amplification

For amplifiers with active regulation (by solution in vitro) «Tercyck» (SPA «DNA–Technology», Russia)				
number cycle	step	temp	time	number of repetitions
1	1	95˚C	1 мин	1
2	1 2 3	95˚C 60˚C 72˚C	10 сек 10 сек 20 сек	30
3	1	72˚C	2 мин	1

Also experimentally concentration of primer to reaction was selected. Too small quantity of primers could lead to occurrence of amplicons of bigger length (more than 1000–2000 b.p.). It is due to the fact that primers in every cycle, annealing on matrix, further do not react, as they are the basis for work of Taq–DNA – polymerase and they are included into amplicon structure. In other words, during lack of limits primers and initial sufficient quantities of matrix DNA *Bacillus cereus* Taq–DNA-polymerase works without restriction area, in other words as much as its activity allows it. But during high concentration primers form dimmers, annealing on themselves or steamed, forming amplicon with length of no more 100 b.p. However, it should be noticed that dimmers usually begin to form at the final cycles of reaction. Therefore, optimal PCR concentration of primers for each reaction, from the point of view of effectiveness was selected. However in this case versatility of the working quantity was necessary. After some conducted experiments optimal universal concentration of each primer was determined – 10 pmol per reaction with volume of 25 mql.

During optimization of PCR protocol electrophoretic method of detection of amplification products was used. In picture 8 the final result of these experiments is shown.



Pic 8: The results of amplification of gene HBL enterotoxin fragment: M – molecular weight marker; 1,2 – positive control; 3 –bacteriophage *FBc – 28 UGSHA - Bacillus cereus*; 4 – negative control

As the result PCR – system for indication of presence of gene HBL enterotoxin fragment was developed. During its appliance of fragments ofvirulent gene enterotoxin bacteria *Bacillus* in bacteriophage genome *Bacillus cereus FBc – 28 UGSHA* wasn't found, it shows its possible usage for decontamination of fod products.

As the result of conducted research sequencing genome data of bacteriophage *Bacillus cereus FBc – 28 UGSHA* were obtained, the map of linear DNA with decoding genome regions were made. According to known analogues expression products of their genes were determined. Qualitative composition of proteins of bacteriophage *Bacillus cereus FBc – 28 UGSHA* correspond to such at annotated analogues, it has distinct homologies of nucleotide and amino acid sets. In structure of proteins regularity is observed, peculiar for given viral particles-presence of structural and non-structural components. Also gene products were found, that didn't have distinct functional characteristics, so called hypothetic proteins, having analogues in annotated bacteriophage genomes, active towards studied bacteria species. It was established that the use of phenol chlorophorm extraction leads to the best exit of matrix DNA. The system of molecular genetic indication (with the use of PCR) of autonomous genetic elements (pathogenic islands) in bacteriophage genomes was developed and they were supposed for appliance as a special mean for decontamination of raw food material and ready for application of meat, fish and diary food products.

CONCLUSIONS

The uniqueness of gene candidate was determined and the fragment, coding gene HBL enterotoxin was chosen. Characteristic of primers to gene HBL enterotoxin regions of phage genome, active towards *Bacillus cereus:* upstream primer (f) 5'-3' – GAGATGCAAAAATTAATGCGGCG; downstream primer (r) 5'-3' – TGCGATTCCTAGCGGAGTTC; Predicted temperature of melting of upstream primer - 60,0 ˚C; Predicted temperature of melting of downstream primer - 59,9 ˚C; Theoretical specificity - *Bacillus cereus*; Length of amplified region (b.p.) – 366. Based on the results of experimental research of indication of specific gene region HBL enterotoxin culture *Bacillus cereus* with developed system of oligonucleotides in bacteriophage genomes *Bacillus cereus FBc – 28 UGSHA* pathogenic locuses were not found.

Obtained data allows to recommend bacteriophage *Bacillus cereus FBc – 28 UGSHA* for construction of new high-effective phage biopreparation with the aim to accomplish mediate bioprocessing (treatment of food raw material and end products by bacteriophages, furthering the increase of expiry date), allowing to eliminate (destroy) pathogenic microorganisms from food raw material of animal origin and ready to serve meat, fish, diary food products.

The research is conducted according to thematic plan of scientific research made by order of epy Ministry of Agriculture of Russian Federation in 2018.

REFERENCES

[1] Scallan E, Hoekstra RM, Angulo Scallan FJ. Foodborne illness acquired in the United States - major pathogens. *Emerg Infect Dis*, 2011; 17(7): 15.

[2] Phelps LS, McKillip JL. Enterotoxin production in natural isolates of *Bacillus* outside the *Bacillus cereus group*. *Appl. Environ. Microbiol*, 2002; 68: 3147-3151.

[3] Pruss BM, Dictrich R, Nibler B, Martibauer E, Scherer S. The hemolytic enterotoxin HBL is broadly distributed among species of the *Bacillus cereus group*. *Appl. Environ. Microbiol,* 1999; 65: 5436-5442.

[4] Gostev VV, Goncharov AE, Gracheva MA, Sidorenko SV. Spread of gene complex immune evasion cluster and other viral factors at *Staphylococcus aureus*. *Clinical microbiology and antibacterial chemo*, 2013; 15 (4): 270-278.

[5] Techexpert. SanRaN 2.3.2.1078-01 Hygienic requirements of safety and nutritional declaration – URL: http://docs.cntd.ru/document/901806306 - access data 12.02.2018.

[6] Kutter E., Sulakvelidze A. Bacteriophages: biology and applications. Boca Raton, FL: CRC Press, 2005. 510 p.

[7] Clokie Martha RJ, Kropinski AM, Lavigne R. Bacteriophages. Methods and Protocols, Volume 3. Humana Press, 2018. 311 p.

[8] Alyoshkin AV, Svetoch EA, Volozhantsev NV, Kiselyova EA, Rubalskiy OE, Yershova ON, Novikova LE. Innovative ways of bacteriophage usage in sanitary and epidemiological welfare of population in the Russian Federation. *Bacteriology*, 2016; 1(1): 22-32.
[9] Zulkarneev Eldar Rinatovich. Development of means of decontamination and increase of expiry date of cold fish on the basis of bacteriophages: dissertation. … PhD in Biological sciences: 03.02.03; 03.01.06. Astrakhan, 2017. p. 14.
[10] Belova KV, Feoktistova NA, Vasilyev DA. Bacteriophages *Bacillus coagulans*: ways of detachment and cultivating parameters. *Vestnik Ulyanovsk State Agricultural Academy,* 2016; 2(34): 80-86.
[11] Gordon R. The genus *Bacillus*. *Handb. Microbiol. Cleveland (Ohio),* 1973; 1: 71–88.
[12] Slepecky RA, Hemphill HT. The Genus *Bacillus*-Nonmedical. *Prokaryotes,* 2006; 4: 530–562.
[13] Feoktistova NA, Vasilyev DA, Zolotukhin SN. The results of benchmarking analysis of bacteriological research methods of cocopowder for baccilla, that cause the food spoilage (BCFS). *Vestnik Ulyanovsk State Agricultural Academy*, 2015; 1(29): 69-76.
[14] Aukenov NE, Masabaeva MR, Khasanova UU. Detachment and cleanness of nucleic acids, problem state on the modern times. *Science and health service*, 2014; 1: 51-53.
[15] Kulmaganbetova GN. Modern biology problems. Astana, 2012, p. 7-8.