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Original article

Determination and Study of Main Biological Features of Bacteriophages Active Against Bacteria *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*

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ABSTRACT

Background and aims*. One of the significant areas of modern microbiology and biotechnology is the study of bacteriophages. This is due to the growing interest in terms of their practical application in various branches of medicine, agriculture, and the food industry. The purpose of our work was to isolate bacteriophages active against bacteria of the genus Pseudomonas and to study their biological properties.* ***Methods.*** *Bacteriophages isolated from spoiled poultry, beef and carp samples were the subjects of the study. 20 g of the weighed sample were inoculated with 20µl of Pseudomonas spp. diluted 24 hrs culture and incubated at 30°C for 2 days, the cultures were infected with phages at a dose of 0.1 ml and continued cultivation for another 4– 5 h until onset of culture lysis.* ***Results.*** *In the studies, 8 bacteriophages were isolated, of which 4 are active against P. fluorescens bacteria (BV-4, BV-5, BV-55, BV-71) and 4 were active against P. aeruginosa bacteria (BV-12, BV-23, BV-25, BV-57). Negative colonies of bacteriophages specific to P. aeruginosa were characterized by the following parameters: diameter 3.0–4.5 mm, completely transparent, without a zone of secondary lysis; and phages specific to P. fluorescens had a diameter of 2.0–4.0 mm, were completely transparent, without a zone of secondary lysis.* ***Conclusions.*** *Protecting protein-containing foods that have not undergone heat treatment from microbial contamination by using strains of bacteriophages BV-25 and BV-55, Showed efficacy against bacteria of the genus Pseudomonas.*

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INTRODUCTION

One of the significant areas of modern microbiology and biotechnology is the study of bacteriophages. This is due to the growing interest in terms of their practical application in various branches of medicine, agriculture, and the food industry [1,2]. Given the significant prevalence and circulation of *Pseudomonas* in nature, much attention is paid to the detection of these microorganisms in various objects. Microbiological studies have shown that bacteria of the genus *Pseudomonas* (*P. fluorescens*, *P. aeruginosa*) play a key role in the spoilage of milk [3,4], meat of slaughter animals and poultry, eggs, and fish [5-7]. *P. fluorescens* and *P. aeruginosa* are psychrophilic, obligate aerobic microorganisms that can multiply in products during refrigerated storage. These bacteria secrete active enzymes that

break down proteins and lipids. They are antagonists of many bacteria and mold. The search for environmentally friendly ways to decontaminate food products to increase their shelf life and reduce spoilage is an urgent task. Also, as the collections of bacteriophages expand, the number of possible ways to use them will also increase. The purpose of our study was to isolate bacteriophages active against bacteria of the genus *Pseudomonas* and to study their biological properties.

METHODS

Study design and setting

Nine strains of bacteria of the genus *Pseudomonas*, which we previously isolated from protein-containing food products [7], were used as indicator cultures. Bacteriophages isolated from spoiled poultry, beef and carp samples were the subjects of the study. 20 g of the weighed sample were inoculated with 20 µl of *Pseudomonasspp*., diluted 24 hrs culture and incubated at 30°C for 2 days. Then the resulting material was homogenized in 10 ml of physiological saline, and cells and coarse particles were precipitated by centrifugation at 6000 min–1 in within 15 min. Chloroform (20:1) was added to the lysate, vigorously shaken for 1 min, left for 20–60 min at room temperature, and then centrifuged again to obtain a clarified lysate.

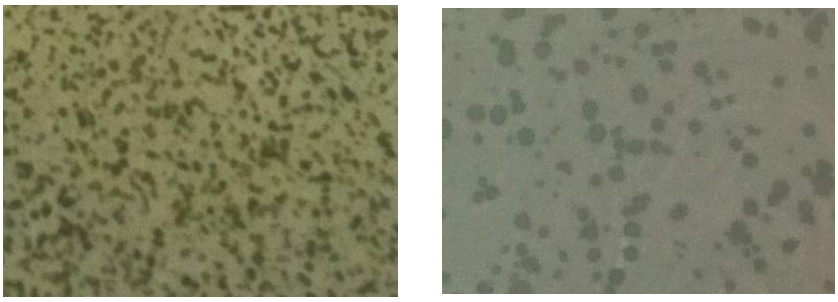
Data collection procedure

The biological properties of the isolated bacteriophages were studied according to the methods proposed by Goldfarb [1], Gabrilovich [8], and Zolotukhin [9]. The bacteriophages yield was determined as follows: 0.5 ml of a daily indicator culture was added to conical flasks with 10 ml of nutrient broth and incubated at 37°C with aeration. After 2 h, at the beginning of the exponential growth phase, the cultures were infected with phages at a dose of 0.1 ml and continued cultivation for another 4–5 h until onset of culture lysis. The titer of phagolysate and the shape of negative colonies of bacteriophages were determined using the agar layer method according to Gracia [10].

Determination of the spectrum of lytic activity and species specificity was carried out by applying lysates to the lawn of a bacterial culture: 0.1 ml of a daily broth culture of the studied bacteria was applied to the surface of nutrient agar in Petri dishes. The suspension was evenly distributed over the surface of the medium with a sterile spatula. left at room temperature under sterile paper filters for drying for 15–20 min. 20 µl of phage lysates were applied to the surface of the inoculated medium and the Petri dishes were tilted so that the drops were drained, and then incubated in a thermostat at a temperature of 30°C. The results were evaluated after 24–48 h to detect zones of lysis and negative colonies.

RESULTS

In the studies, 8 bacteriophages were isolated, of which 4 are active against *P. fluorescens* bacteria (BV-4, BV-5, BV-55, BV-71) and 4 are active against *P. aeruginosa* bacteria (BV-12, BV-23, BV-25, BV-57).Negative colonies of bacteriophages specific to *P. aeruginosa* were characterized by the following parameters: diameter 3.0–4.5 mm, completely transparent, without a zone of secondary lysis; and phages specific to *P. fluorescens* had a diameter of 2.0–4.0 mm, were completely transparent, without a zone of secondary lysis(Figure 1).



A ***B***

Figure 1. Morphology of negative colonies of phages BV-55 (a) and BV-25 (b)

Table 1, presents the results of experiments to determine the yield and titer of isolated bacteriophages.The spectrum of lytic activity of phages was determined using 9 bacterial strains, 6 of which belonged to *P. fluorescens* and 3 to *P. aeruginosa*.

Table 1. Titer of phages specific to bacteria of the genus Pseudomonas

Phage strains	BV-4	BV-5	BV-55	BV-71	BV-12	BV-23	BV-25	BV-57
Titer (PFU/ml)	$1{,}9\times10^{10}$	$8{,}0\times10^{9}$	$2{,}6\times10^{10}$	$5{,}0\times10^{9}$	$1{,}3\times10^{10}$	$8{,}0\times10^{9}$	$9{,}0\times10^{9}$	$1{,}5\times10^{10}$
Average yield (PFU/%)	110	25	82	5	102	88	104	55

It was established that the BV-25 phage strain had the widest spectrum of lytic activity in relation to the studied cultures, which lyses 5 out of 9 tested strains: *P. aeruginosa 224, 410, 150* and *P. fluorescens 112, 320. 25* is characterized by a fairly wide spectrum of lytic action, and besides, it has cosmopolitanism: it is able to infect two types of bacteria.BV-55 infects 4 out of 9 strains tested: P. *fluorescens 112, 320, 220* and *P. aeruginosa 150* (Table 2).

Table 2. Spectrum of lytic action of phages specific to bacteria of the genus Pseudomonas

Phage strains	Spectrum of lytic action	Percentage lysed of bacterial strains
BV-4	*P. fluorescens-112, 130, 311*	33,3%
BV-5	*P. fluorescens-320, 220, 222*	33,3%
BV-55	*P. fluorescens-112, 320, 220* *P. aeruginosa-150, Azotobacter*	44,4%
BV-71	*P. fluorescens-112, 320, 222*	33,3%
BV-12	*P.aeruginos-410, 150*	22,2%
BV-23	*P.aeruginosa-224, 410, 150*	33,3%
BV-25	*P. aeruginosa-224, 410, 150* *P. fluorescens-112, 320* *Azotobacter* *Escherichia col*	55,5%
BV-57	*P.aeruginosa-224, 410, 150*	33,3%

DISCUSSION

The current study showed that the processing of samples of protein-containing products with suspensions of bacteriophages of bacteria of the genus *Pseudomonas* protects these products from contamination. The combined use of three bacteriophages increased the shelf life of all samples tested at 4°C and poultry and beef samples at 30°C. The samples treated with phage suspension and the samples treated with phage suspension plus host cells demonstrated almost the same results – mucous appeared on the samples surface only on day 6 – 8, simultaneously with changes in color and odor.

According to Eller, *et al* 2014 and Lu, *et al* 2017 in naturephages with activity against P. fluorescens strains were isolated more frequently from the samples of river and stream water and dairy industry wastewater [11,12]. Phages infecting P. fluorescens also have been isolated from various environments, including soil, water, sewage, and effluents and wastewater from dairy industries, this makes them a good choice for preserving different foodstypes. Other study done by Nascimento *et al.,* 2022 showed the phages isolated in the study have shown great potential to be applied for biocontrol of *Pseudomonas* and prevent spoilage at raw milk stored under refrigeration [13]. By reducing the bacterial count, they inhibited or delayed protease production and casein hydrolysis, directly affecting the quality of dairy products. The phages reported here have exciting characteristics such as thermal resistance and activity over a wide pH range, which allow their application for diverse purposes in the dairy industry.

The current results along with previous literatures that provided similar results suggested the use of natural killer of bacteriaphages is something that can bring great benefit to mankind, represented in preserving food by reducing bacterial growth on it in natural ways.

CONCLUSION

Protecting protein-containing foods that have not undergone heat treatment from microbial contamination by using strains of bacteriophages BV-25 and BV-55, Showed efficacy against bacteria of the genus *Pseudomonas*. Protein-contained food treatment with suspensions of bacteriophages resulted in twofold increase of food shelf life at 4°C as well as at 30°C. We recommend more researches on this subject using other foods models, to evaluate the effectiveness bacteriophages against bacteria of the genus *Pseudomonas*.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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