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Study of *Serratia* phages using virulence tests

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Abstract

One of the main tests in selection of therapeutic phages is studing their virulence. Because of high stability of lysogenic status of bacterial culture, as well as ability in some cases of horizontal transfer of prophages and antibiotic resistance factors, existence of the moderate phages in medical preparations may cause negative results. The *Serratia* phages were studied with two main tests in the present work: sowing effectiveness and range of lysis action, as well as phage reproductive capacity on the host cells irradiated with UV rays. The results obtained showed that sowing effectiveness of studied phages and range of action are quite high. Out of 14 clones of *Serratia* phages 11 were characterized with reproductive ability on the host strains inactivated by UV rays. The results of above experimental works and other earlier tests allowed us selecting the components of *Serratia* phages for the medical-preventive preparation.

Keywords: *Serratia marcescens*; Bacteriophage; Virulence; Strain; Medical-preventive preparations.

1. Introduction

As evidenced by experimental data, in the course of our research, we selected phages specific to conditionally pathogenic strains of *Serratia marcescens*. A number of parameters necessary for the primary selection of each new phage with potential therapeutic properties have been established. However, for a drug that meets modern requirements, it was necessary to conduct a series of tests to determine the therapeutic potential of the phage.

The most important aspect in the selection of therapeutic phages is determining phage virulence criteria. The classical concept, based on a well-known concept in medicine, states that virulence is the determination of the degree of pathogenicity. However, in some cases, this determination is based on studying various stages of virus-cell interaction. Although, in these processes, the stability factor of the experiment, which depends on a number of primary and secondary factors, has not been calculated [5].

The division of phages into virulent and temperate ones is crucial for science and, at the same time, is the main test in the design of therapeutic phages.

It is known that virulent phages, while infecting bacteria, enter the vegetative phase of reproduction, forming mature progeny particles, and then lyse the host cell. Temperate phages are capable of causing both a lytic reaction and a lysogenic reaction, in which the infecting phage does not multiply but enters the prophage state, allowing the host cell to survive by becoming lysogenic. Lysogenicity is a stable characteristic of a bacterial cell. According to a number of scientists, during lysogenization, the sequential functioning of a group of genes occurs, alternately suspending or restoring the synthesis of cellular DNA. The transition of the prophage to a vegetative state determines the process of induction of a temperate phage, i.e., reproduction of a temperate phage [1, 6, 8].

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Lysogenic strains are immune to phages related to their prophage, but at the same time, they are not resistant to other types of phages (heteroimmune infections). As a result, strains can contain multiple prophages and become multiply resistant to phages, which significantly reduces the effectiveness of phage therapy. The immunity associated with the lysogenic state does not serve as an obstacle to the adsorption of the phage on the cell. Therefore, this immunity must be distinguished from phage resistance, which arises as a result of a change in the cell membrane that blocks the adsorption of the phage on the cell. Considering the high stability of the lysogenic state of the culture, their ability to horizontally transfer prophages, and the ability in some cases to transfer antibiotic resistance factors, one can imagine the damage that the presence of temperate phages in the composition of the therapeutic drug can cause to phage therapy [3].

In this work, the results of two main tests that determine the truly virulent nature of bacteriophages are studied and analyzed. These tests include the inoculation efficiency and lytic range of the bacteriophages, as well as their ability to multiply on UV-inactivated host strains.

2. Material and methods

The following materials and reagents were used:

- Bacterial strains: *S. marcescens*;
- 14 phage clones of *S. marcescens*;
- Media and reagents: Bacto agar; Brain Heart Infusion Broth; Brain Heart Infusion agar; Beef Extract Powder; HRM (Hydrolyzed Fish Meal) broth; GRM agar; peptone enzymatic;
- Sterilizing membrane filters, Chamberlan ceramic filters;
- Ultraviolet irradiator with a mercury element, emitting a wavelength of 2537 Å.

2.1. Seeding Efficiency and Lytic Range

The efficiency of inoculation was determined by the ratio of the phage titer on the test strain to the titer of the same phage on the host strain.

Sowing efficiency was calculated using the formula:

$$E = \text{Inoculation efficiency} = \frac{\text{phage titer on the research strain}}{\text{phage titer on host strain}} = \frac{T}{T_0}$$

The range of lytic activity of the phage was determined by the Craigie method. Cultures were plated in the form of strips. After drying the cultures in a thermostat for 15-20 minutes, bacteriophage was applied to the strips and incubated in a thermostat at 37°C for 18 hours.

2.2. Bacteriophage Performance on UV-Inactivated Cells

To determine the UV irradiation required for complete inactivation of bacterial cells, aliquots of a bacterial emulsion containing $5 \cdot 10^8$ cells/ml were treated with a BUF-15 lamp at different exposures. Then, 1 ml of phage containing 10^5 parts/ml was added to the completely inactivated bacterial emulsion. The mixture was incubated at 37°C, and 1 ml samples were taken at various intervals and titrated using the two-layer method. Productivity was determined by comparing the results of propagation of a given bacteriophage on non-inactivated cells [2, 4, 7].

3. Results and Discussion

As our studies have shown, the efficiency of inoculating *S. marcescens* on the host's own strain is equal to one. If this indicator is similar for other strains, then these phages have a high range of lytic activity, which indirectly indicates the virulence of these phages. The results of the inoculation efficiency of *S. marcescens* phages are shown in tables 1, 2, 3, 4.

Experiments to determine the virulence properties of phages were carried out in two directions. In the first series of experiments, the doses and irradiation conditions for complete inactivation of host cells were determined. Since the host cell for *Serratia* phages in our main experiments was the bacterial strain *S. marcescens* 285, experiments were carried out on this strain.

A 24-hour agar culture was washed with saline and diluted to $5 \cdot 10^8$ viable cells per ml. 0.1 ml was taken from the main tube, and all successive dilutions were inoculated on Petri dishes with nutrient agar to determine the exact number of viable bacteria before irradiation (culture control). At the same time, a control experiment of phage reproduction was carried out on a non-irradiated culture (phage control).

The initial washout from the culture was transferred to a sterile glass Petri dish and placed for irradiation at a distance of 30 cm under a UV lamp with a wavelength of 2537 Å.

Table 1 Results of determining the efficacy of SM1N phage culture on *S. marcescens* strains

№	Name of the strain *S. marcescens*	Title by Grace	The Nature of Negative Colonies	Efficiency Sowing
1	285 Strain Host	$5x10^8$	Middle colony with red clear center and muddy halo	1
2	282	$1x10^7$	Small Clear Colonies	0,02
3	155	$2x10^7$	Middle colony with clear center and halo	0,04
4	224	$2x10^7$	Medium colonies transparent	0,04
5	225	$3x10^7$	Medium colonies transparent	0,06
6	409	$2x10^7$	Colonies with a murky halo	0,09
7	544	$3x10^6$	A large colony with a transparent center	0,006
8	586	$2x10^7$	Medium colony with a clear center and a shallow murky halo	0,04
9	615	$2x10^7$	Medium colony with point center	0,04
10	658	$1x10^7$	Medium colony with large center and halo	0,02
11	754	$3x10^7$	Medium Turbid Colony	0,06

Table 2 Results of determining the efficacy of SM2N phage culture on *S. marcescens* strains

№	Name of the strain *S. marcescens*	Title by Grace	The Nature of Negative Colonies	Efficiency Sowing
1	285 Strain Host	$2x10^8$	Medium transparent colony with a shallow, clear center	1
2	282	$2x10^8$	Medium clear colony with shallow center	1
3	3	$2x10^8$	Large transparent colonies	1
4	108	$3x10^7$	Large Clear Colonies	0,15
5	275	$4x10^7$	Medium Clear Colonies	0,2
6	241	$2x10^8$	Large Clear Colonies	1
7	405	$5x10^6$	Medium Clear Colonies	0,025
8	430	$7x10^7$	Large Clear Colonies	0,35
9	483	$1x10^8$	A large colony with a large halo, punctate and turbid punctate center	0,5
10	638	$6x10^7$	Medium colony with large clear center and halo	0,3
11	658	$8x10^7$	A large colony with a large, clear center and a shallow halo	0,4

Table 3 Results of determining the efficacy of SM3N phage culture on *S. marcescens* strains

№	Name of the strain *S. marcescens*	Title by Grace	The Nature of Negative Colonies	Efficiency Sowing
1	285 Strain Host	$3x10^8$	Medium colony with shallow center and halo	1
2	282	$2x10^8$	Medium colony with shallow center and turbid halo	0,7
3	40	$7x10^7$	Small Clear Colonies	0,23
4	108	$3x10^6$	Large transparent colonies with a shallow cloudy center	0,02
5	142	$1x10^8$	Large Murky Colonies	0,3
6	155	$1x10^8$	Medium turbid colonies with center and halo	0,3
7	224	$3x10^7$	Large colonies with a murky shallow center	0,1
8	225	$4x10^7$	Medium colony with a cloudy halo and a clear center	0,13
9	638	$4x10^7$	A large colony with a large center	0,13
10	658	$9x10^7$	Medium colony with transparent center and cloudy halo	0,3
11	754	$5x10^7$	Medium colony with shallow center and halo	0,17

Table 4 Results of determining the efficacy of SM4N phage culture on *S. marcescens strains*

№	Name of the strain *S. marcescens*	Title by Grace	The Nature of Negative Colonies	Efficiency Sowing
1	285 Strain Host	$5x10^8$	Large colonies with a small center	1
2	282	$1x10^8$	Medium colony with center and halo	0,2
3	142	$7x10^7$	Medium colony with a shallow, clear center	0,4
4	155	$2x10^8$	Medium colony with a clear center	0,6
5	224	$3x10^8$	Medium colony with a clear center	0,2
6	241	$1x10^8$	Medium Clear Colonies	0,16
7	415	$4x10^8$	Large colonies with a clear center	0,5
8	736	$5x10^7$	Medium colony with large transparent center	0,1
9	754	$2x10^8$	Large clear colonies with a murky center	0,4
10	719	$1x10^8$	Medium Colonies	0,2
11	544	$2x10^7$	Large colonies with a clear center	0,04
12	619	$6x10^7$	Medium colony with large transparent center	0,12

After irradiation of the strain, the cup was closed with a glass lid, and the survival of the strain was simultaneously monitored in complete darkness. Inoculation of the studied phage in the ratio of 1 ml of irradiated culture and 1 ml of phage in a titer of $5 \cdot 10^5$ viable virions per ml was carried out on fully inactivated cells. Phage samples were taken from experimental and control tubes after 0 minutes, 2 hours, 6 hours, and 18 hours after the beginning of the experiment, and the number of phage particles in the experimental and control samples was determined. The results of experiments to determine the virulence properties of 12 potentially therapeutic phages of *S. marcescens* are presented in Table 5.

Table 5 General scheme of reproduction *of Serratia* bacteriophages on bacteria, inactivated by UV rays

№	Initial Bacterial Count Sm285, cl/ml	Phage Name *Serratia*	Original Quantity phages particle/1ml	Dynamics of phage increase on UV inactivated bacterial cells				
				0hrs	2hrs	6hrs	18hrs	Control phages
1	$3x10^8$	SA1	$1x10^6$	$2x10^5$	$21x10^5$	$2x10^6$	$8x10^7$	$1x10^8$
2	$3x10^8$	SA3	$3x10^6$	$5x10^5$	$9x10^5$	$3x10^6$	$3x10^8$	$3x10^8$
3	$3x10^8$	SM6	$3x10^6$	-	$5x10^5$	-	$1x10^4$	$3x10^8$
4	$4x10^8$	SM3	$1x10^6$	$13x10^5$	$15x10^5$	$1x10^6$	-	$7x10^8$
5	$5x10^8$	SM4	$3x10^6$	$9x10^5$	$25x10^5$	$2x10^6$	$1x10^8$	$3x10^8$
6	$5x10^8$	SMIN	$2x10^6$	$6x10^5$	$18x10^5$	$20x10^6$	$18x10^8$	$2x10^9$
7	$5x10^8$	SM2N	$2x10^6$	$3x10^5$	$2x10^6$	$3x10^6$	$20x10^8$	$2x10^9$
8	$5x10^8$	SM3N	$1x10^6$	$5x10^4$	$1x10^5$	$1x10^6$	$30x10^8$	$1x10^9$
9	$5x10^8$	SM4N	$2x10^6$	$5x10^5$	$6x10^5$	$9x10^5$	$30x10^8$	$2x10^9$
10	$4x10^8$	SM5	$1x10^6$	$4x10^5$	$1x10^6$	$3x10^6$	$3x10^8$	$3x10^8$
11	$3x10^8$	SM3	$1x10^6$	$5x10^5$	$4x10^5$	$2x10^6$	$15x10^8$	$5x10^8$
12	$3x10^8$	SM4	$3x10^6$	$2x10^5$	$9x10^5$	$1x10^6$	$3x10^8$	$3x10^8$
13	$3x10^8$	SM5N	$1x10^6$	$10x10^5$	$16x10^5$	$20x10^5$	$9x10^6$	$1x10^9$

As can be seen from the data presented in the table, out of the 14 *Serratia* phage clones we tested, 11 showed the ability to reproduce on strains completely inactivated by UV rays. However, 2 phages, SM3 and SM6, did not exhibit the ability to reproduce on host cells whose genetic material was subject to destruction under the influence of UV rays.

4. Conclusion

The conducted experiments allowed us to differentiate between phages, which we have already selected as potentially therapeutic, into true virulent phages and apparently non-aggressive virulence phages. The latter property, in our opinion, is especially important in the selection of therapeutic phages.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest amongst the authors.

References

[1] Bujak, K., Decewicz, P., Kaminski, J., & Radlinska, M. (2020). Identification, characterization, and genomic analysis of novel Serratia temperate phages from a gold mine. *International Journal of Molecular Sciences*, *21*(18), 6709.

[2] Clokie, M. R., Kropinski, A. M., & Lavigne, R. (2009). *Bacteriophages* (Vol. 501). Leicester, UK:: Humana Press.

[3] Guo, Z., Lin, H., Ji, X., Yan, G., Lei, L., Han, W., & Huang, J. (2020). Therapeutic applications of lytic phages in human medicine. *Microbial pathogenesis*, *142*, 104048.

[4] Hyman, P., & Abedon, S. T. (2009). Practical methods for determining phage growth parameters. *Bacteriophages: methods and protocols, volume 1: isolation, characterization, and interactions*, 175-202.

[5] Khan Mirzaei, M., & Nilsson, A. S. (2015). Isolation of phages for phage therapy: a comparison of spot tests and efficiency of plating analyses for determination of host range and efficacy. *PLoS one*, *10*(3), e0118557.

[6] Luria, S. E., Darnell, J. E., Baltimore, D., & Campbell, A. (1978). General virology. In *General virology* (pp. 578-578).

[7] Pelzek, A. J., Schuch, R., Schmitz, J. E., & Fischetti, V. A. (2013). Isolation, culture, and characterization of bacteriophages. *Current Protocols Essential Laboratory Techniques*, *7*(1), 4-4.

[8] Tian, C., Zhao, J., Zhang, Z., Chen, X., Wei, X., Li, H., ... & Zhao, X. (2019). Identification and molecular characterization of Serratia marcescens phages vB_SmaA_2050H1 and vB_SmaM_2050HW. *Archives of virology*, *164*, 1085-1094.