Short communication

INFLUENCE OF THE ENHANCERS ON THE BACTERIOPHAGES ADAPTATION TO PSEUDOMONAS AERUGINOSA CLINICAL STRAINS

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A characteristic feature of the current state of the fight against infectious diseases is the search for alternative to antibiotics. This is due to the fact that the majority of pathogen bacteria have acquired new mechanisms resistance to existing antibacterial drugs [1,2 ,3].

One of the areas of treatment of infectious patients can be considered bacteriophage therapy. Opinion of many doctors and scientists to bacteriophages therapy is ambiguous. Since the 30-s last century, there has been a negative opinion, and the promotion of the miraculous effectiveness of phages. There are reasons for this, the analysis of which requires a separate study.

The accumulation of new knowledge in the immunology and molecular biology fields makes it possible to significant improve the composition of bacteriophage preparations. Leading infectiologists predict that phage therapy will soon become highly effective in combating infectious diseases [4, 5].

Of particular importance is the production of highly virulent phages capable of withstanding significant nosocomial or epidemically strains of pathogens.

It is known that phages have a high specificity, have a different level of lytic activity, and are able to pass into a state of prophage, or adapt to the circulating bacteria strain and acquire the ability to cause their lysis [6].

The purpose of this work was to obtain highly virulent bacteriophages by adapting their moderately sensitive clones to clinical strains of *P. aeruginosa*.

The objective of these studies was to experimentally determine the feasibility of using some enhancers capable of stimulating growth and reproduction rate of pseudomonads, which affects the reproduction of bacteriophages and their adaptation to specific populations of bacteria. As a review of literature shows, many scientific studies in recent years have been devoted to the study of the stimulating effect of various physical and chemical factors on the biological properties of cells.

A particularly important area in microbiology is the search the substances that can significantly accelerate of colony formation, the mass accumulation of biotechnologically significant microorganisms and stimulate the production by cells of useful compounds - the so-called enhancers [7].

This is very important for the development of cost-effective technologies for the production of microbial synthesis products for the needs of medical microbiology, biotechnology, pharmacy, etc.

Another important motive for developing the technique of increasing the lytic activity of specific phages, through their adaptation, is their use for the production of vaccine preparations by phagolysis.

Materials and methods

The object of study was the culture of 15 strains of *P. aeruginosa* (strain ATSS 27853, strain 66-16 - sensitive to phage, and 13 freshly isolated clinical strains).

For experimental studies used commercial preparations of bacteriophages (*P. bacteriophage* (Microgen, Perm, Russia)).

As enhancers, were used: 1- (3,4-dimethoxybenzyl) -6,7-dimethoxyisoquinoline (0.5 ml), 2.2 ', 2' ', 2' '- (4,8-di (piperidin-1 -yl) pyrimido [5,4-d] pyrimidine-2,6-diyl) bis (azanetriyl) tetraethanol (4 ml), 2- (Phenylmethyl) -1H-benzimidazole (1 ml), which previously showed the ability to synergize to activate microbial growth [12].

For each culture was prepared for 5 tubes with the following composition:

tube number 1 - 1 ml of culture (10^7 CFU / ml) + 1 ml 0,9 % NaCl; tube N. 2 - 1 ml of culture (10^7 CFU / ml) 1 ml of medium with enhancers; test tube N. 3 - 1 ml of culture (10^7 CFU / ml) 0.2 ml of pyonogenic phage + 0.8 ml 0,9 % NaCl; test tube N. 4 - 1 ml of culture (10^7 CFU / ml) 0.2 ml of the pyocyanic phage + 0.8 ml of medium with enhancer; test tube N. 5 - 1 ml of culture (10^7 CFU / ml) 0.5 ml of the pyocyanic phage + 0.5 ml of medium with enhancer. Determination of sensitivity to specific bacteriophages was performed by the drip method [8].

The adaptation process included sequential bacteriophage passages in *P. aeruginosa* cultures, obtaining phage filtrate and release from culture by centrifugation at 5000 rpm.

With the "filtrates" obtained in this way, which contained adapted phages, we set tests for determining the phage sensitivity to pseudomonad cultures. In parallel, as a control, we determined the sensitivity of the cultures taken from the original (commercial) bacteriophages. The following passages were carried out until sterile stains were obtained without colony formation secondary growth. The control of the filtrate for the presence of living *P. aeruginosa* cells was carried out by seeding on a solid medium (Mueller-Hinton medium) according to the classic method with the possibility of counting the colonies grown.

We are also used non-parametric statistic methods for obtained results (Xi-square)

Results & discussion

We are conducted a study for sensitivity of phages (commercial preparations of bacteriophages) to pseudomonads regional clinical strains. We have

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established their lack of effectiveness (no more than 50% of *P. aeruginosa* strains were sensitive to the lytic action of pseudomonas bacteriophage) [9].

Comparative analysis of the efficiency of phage adaptation by a known method with using enhancers showed that with the addition of enhancers and phages in pseudomonad cultures, a not significant difference ($p > 0.05$) in bacterial concentration (CFU), and in the passages number, that required achieving complete lysis of the cultures.

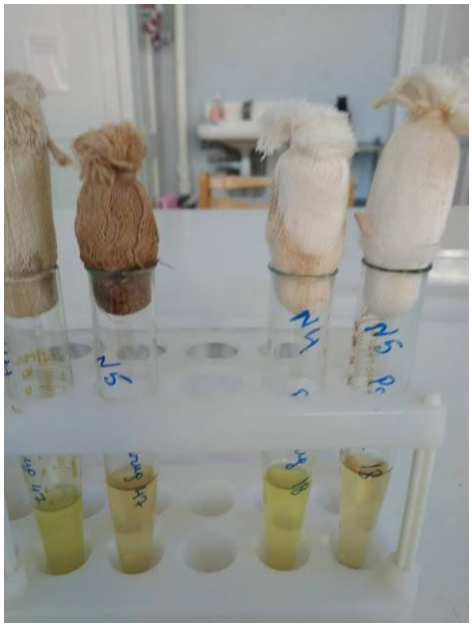

Fig 1. Tubes with *P. aeruginosa*, enhancers and bacteriophage: from left to right: tubes 4 and 5 are cultures of *P. aeruginosa* with the original bacteriophage (4 h incubation), two tubes on the right - additionally with enhancers (2 h incubation). After 2 hours of incubation with enhancers, the amount of pyocyanin decreases, which indicates an increase in the number of active bacteriophages.

This can be explained by rapid adsorption of phage particles on both live, reproducible bacterial cells, and on the dead cells. Moreover, such adsorption is irreversible, and phagolysis is possible only on cultures that are in the exponential growth phase. That is, when a certain part of the culture under the action of enhancers begins to growth rapidly, adsorbed phages are not enough to completely infect them.

Considering the above, we somewhat changed the experiment. Samples with bacteriophage enhancers were added not only simultaneously, but after 2 and 4 hours. When seeding cultures from such samples after 24 hours, the number of CFU / ml was significantly lower ($p < 0.05$), and for full adaptation of the phage of different strains, 3 to 5 passages were performed, while without the use of enhancers – 5-8 passages. The total number of strains that became sensitive to phages under the action of adapted phages was 47%, and with enhancers – 67%. In 20.0 % cases, there was a slight growth of secondary colonies (grade "+++") after the 10th passage, and 2 cultures remained resistant to phages, both to the original and adapted by both methods.

Thus, by adapting phages to clinical strains of *P. aeruginosa*, it was possible to increase their lytic activity by 3.5–5 times, which indicates the promise of using this method to obtain highly effective phage preparations and phagolytic vaccines.

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Introduction. One of the areas of treatment of infectious patients can be considered bacteriophage therapy. It is known that phages have a high specificity, have a different level of lytic activity, and are able to pass into a state of prophage, or adapt to the circulating bacteria strain and acquire the ability to cause their lysis. The purpose of this work was to obtain highly virulent bacteriophages by adapting their moderately sensitive clones to clinical strains of *P. aeruginosa*. **Materials and methods.** The object of study was *P. aeruginosa* 15 strains and 13 freshly isolated clinical strains. For experimental studies used commercial preparations of bacteriophages (Microgen, Perm, Russia)). As enhancers, were used: 1- (3,4-dimethoxybenzyl) -6,7-dimethoxyisoquinoline (0.5 ml), 2.2 ', 2' ', 2' '- (4,8-di (piperidin-1 -yl) pyrimido [5,4-d] pyrimidine-2,6-diyl) bis (azanetriyl) tetraethanol (4 ml), 2- (Phenylmethyl) -1H-benzimidazole (1 ml), which previously showed the ability to synergize to activate microbial growth. Determination of sensitivity to specific bacteriophages was performed by the drip method. The adaptation process included sequential bacteriophage passages in *P. aeruginosa* cultures, obtaining phage filtrate and release from culture by centrifugation at 5000 rpm. **Results and discussion**. The total number of strains that became sensitive to phages under the action of adapted phages was 47%, and with enhancers – 67%. In 20.0 % cases, there was a slight growth of secondary colonies (grade "+++") after the 10th passage, and 2 cultures remained resistant to phages, both to the original and adapted by both methods. **Conclusion**. Thus, by adapting phages to clinical strains of *P. aeruginosa*, it was possible to increase their lytic activity by 3.5–5 times, which indicates the promise of using this method to obtain highly effective phage preparations and phagolytic vaccines.

Keywords: pseudomonas aeruginosa, phages, stimulation of bacterial growth, enhancing of phages adaptation

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