

REVIEW
of the official opponent of a dissertation submitted for a degree of Doctor of
Philosophy in Biology
by Wang Yueqi
on the topic: "Features of the structural organization and interaction of tailed
bacteriophages with biofilm-forming bacteria"
in the specializations 1.5.2. Biophysics and 1.1.10. Biomechanics and
Bioengineering

Relevance of the chosen topic.

The first quarter of the 21st century is characterized by significant changes in the structure of human infectious pathology, which is associated with the widespread distribution of strains of pathogenic bacteria with multiple resistance to antibiotics. Pathogens belonging to the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and species of the genus *Enterobacter*), which are characterized by multidrug resistance to antibiotics, have been classified by the World Health Organization as priority pathogens posing the greatest threat to human health. Currently, the main attention of specialists in microbiology and pharmaceuticals is focused on developing methods to combat these pathogens. However, despite the importance of the problem, the development of new antibiotics is increasingly slowing down, due both to limitations imposed by the targets used and to the fact that existing resistance mechanisms make bacteria less vulnerable even to new substances targeting old targets. In this regard, the problem of using alternative, non-antibiotic means to combat bacteria becomes acute.

One such approach is the use of bacteriophages. In the USSR and later in the Russian Federation, there is a large body of knowledge in the use of bacteriophages as a therapeutic agent to combat pathogenic bacteria. Clinically tested mixtures of bacteriophages have been used in clinical practice for over 50 years. However, to date, they have not become a substantial replacement for antibiotics. This is due to a number of fundamental limitations, including the rather narrow specificity of phages, the rapid development of bacterial resistance that can develop directly during therapy, and the formation of biofilms by bacteria.

Biofilms – three-dimensional structures that include bacterial cells and the polymer matrix they produce – hinder the access of phage particles to cells and can also adsorb phage particles. The latter circumstance is especially relevant for ESKAPE pathogens, which, in addition to antibiotic resistance, are characterized by their ability to form biofilms both in the environment and during infection. Nevertheless, due to the aforementioned exhaustion of the potential of antibiotics, interest in using phages as a means of antimicrobial therapy is constantly increasing. Overcoming the problems associated with phage application requires fundamental research and the efforts of specialists from various fields.

All of the above determines the relevance of the topic chosen by the dissertant.

Degree of validity of the propositions put forward for defense, scientific conclusions, and recommendations formulated in the dissertation

The validity of the propositions put forward for defense, scientific conclusions, and recommendations formulated in the dissertation is determined by the experimental evidence for all statements made by the author. Thus, the architecture of the protein complex in the head-tail region of the *A. baumannii* TaPaz phage with the distribution of the electrostatic surface potential of portal amino acids was demonstrated using high-resolution cryo-electron microscopy (3.18 Å), sufficient to resolve protein structures and build an atomic model. Evidence for the formation of early phage infection vesicles (EPIVs) during the early stages of phiKZ phage infection was obtained by the author using tomography, which yielded high-quality images. The assumption about the use of the host bacterium's inner membrane during EPIV formation follows from the experimental setup and the results obtained.

The study of the interaction of bacteriophages with biofilms formed by clinical isolates of *Pseudomonas aeruginosa* was carried out using scanning electron microscopy, which allowed conclusions to be drawn about structural changes in biofilms caused by the death of part of the cell population under the influence of the phage and the release of DNA. Determination of changes in biofilm biomass was performed using the standard crystal violet staining method. Together with scanning electron microscopy, these two methods confirmed the partial destruction of biofilms by bacteriophages and also revealed the existence of strain-specific differences in the susceptibility of bacteria within biofilms to bacteriophages,

depending on the degree of matrix looseness and pH of the medium. Perhaps, to strengthen the conclusion about the antimicrobial activity of phages against bacteria in biofilms, the author should also have performed plating – this would have made it possible to differentiate the direct reduction in the number of live bacterial cells in biofilms from the destruction of the matrix.

In the process of evaluating the possibility of using silk fibroin as a carrier for the prolonged release of phage particles, the author used a set of methods including Raman spectroscopy, X-ray spectroscopy, scanning electron microscopy, and phage titer determination. The antibacterial activity of the functionalized substrates was proven by the author on agar nutrient media and in nutrient broths, and the absence of cytotoxicity was shown on fibroblast cultures.

Overall, the combination of different methods created an evidence base for the conclusions, and the comprehensiveness of the research declared in the propositions for defense is beyond doubt.

Reliability of the propositions put forward for defense, scientific conclusions, and recommendations formulated in the dissertation

The reliability of the obtained results is due to the set of objective methods used to assess the research parameters, the reproducibility of the obtained results, and the application of statistical processing methods.

Novelty of the propositions put forward for defense, scientific conclusions, and recommendations formulated in the dissertation

The work obtained a number of original results of scientific novelty. In particular, the author visually demonstrated the initial stages of "nucleus" formation by giant "nucleus-forming" phages. The author for the first time proved a link between the three-dimensional structure of biofilms and their sensitivity to phage exposure, showing that a loose biofilm structure makes it more susceptible to bacteriophage activity. The author also was the first to demonstrate the effectiveness of using phage-functionalized fibroin carriers against pathogenic bacteria. Overall, the obtained results contribute to the development of comprehensive methods for combating biofilms using bacteriophages.

Compliance of the dissertation content with the specialization

The content of the dissertation by Wang Yueqi corresponds to specialization 1.5.2. Biophysics, namely its following directions:

1. Theoretical and experimental study of the principles of structure and physicochemical mechanisms of functioning of living systems at all levels of their organization – from molecular and cellular to biospheric (4) Biophysics of complex systems: Biophysical ecology.
2. Physical mechanisms of crucial biological processes, including quantum-mechanical, thermodynamic, and symmetry aspects of interactions determining the most important life processes – structure formation, energy conversion, matter, and information. Molecular motors and machines.
3. Theoretical and experimental study of physical processes occurring in biological systems at different levels of organization, including the study of the effects of various types of radiation and other physical factors on biological systems.

Also, the content of the dissertation corresponds to certain directions of specialization 1.1.10. Biomechanics and Bioengineering, namely points:

3. Study of the mechanics of cell and subcellular structure movement (membranes, cytoskeleton, cytoplasm, cilia, etc.), including mitotic movements, phagocytosis, vesicular transport.
4. Study of the mechanical foundations and manifestations of growth, development, and adaptation processes of biological objects.

Remarks and questions on the dissertation.

Overall, there are no significant remarks regarding the design and content of the dissertation. However, upon reading, several questions arose, including:

1. The dissertation suggests that the formation of EPIVs is associated with the invagination of the cytoplasmic membrane. Can it be assumed that the mechanical process of phage DNA injection is the cause of the invagination?
2. The dissertation demonstrates noticeable changes in biofilm morphology stimulated by the addition of the phage. In your opinion, are these changes mainly associated with the death of part of the cell population due to phage activity, or are there other mechanisms? For example, can components or whole phage particles be incorporated into the matrix?

3. Is the mechanism of phage particle release from the fibroin scaffold only associated with silk degradation, or are there other mechanisms of particle release? These questions do not diminish the significance of the dissertation research. The dissertation meets the requirements established by Lomonosov Moscow State University for works of this kind. The dissertation research has been prepared in accordance with the requirements of the Regulation on the Council for the Defense of Dissertations for the Academic Degree of Doctor of Philosophy, for the Academic degree of Doctor Habilitatus at Lomonosov Moscow State University. The candidate Wang Yueqi deserves to be awarded the degree of Doctor of Philosophy in Biology.

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12.05.2026

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Specialization in which the official opponent defended their dissertation:
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