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Doctoral dissertation

THE INFLUENCE OF SELECTED ANTIMICROBIAL FACTORS ON BACTERIOLYTIC ACTIVITY OF BACTERIOPHAGES

Abstract

The main objective of the doctoral dissertation was to determine the effect of selected antimicrobial agents on the bacteriolytic activity of bacteriophages, taking into account the interactions between these agents. The presence of interactions of various nature - synergistic and antagonistic, as well as the absence of significant interactions - was predicted.

As part of the dissertation topic, three research trends were undertaken, corresponding to three selected types of antimicrobial agents. These trends included antibiotics, nanomaterials and plant extracts combined with lytic bacteriophages. The chosen multidirectional research approach was aimed at determining whether the given antimicrobial agents have co-application potential with lytic phages, i.e. whether there is a possibility of their simultaneous application as part of combined antibacterial therapy.

With the discovery of the phenomenon of synergistic action of some antibiotics and lytic phages (phage-antibiotic synergy, PAS), the scientific community became interested in research on the interactions of the above-mentioned factors, which resulted in the appearance of a large number of scientific papers on this subject in a relatively short time. While studying the literature in terms of planning research for presented doctoral dissertation, the lack of unification of the method for the initial detection of PAS was noticed - in many papers the technique of double-layer agar on Petri dishes (DLA) was used, but the method of application of antibiotics differed in its' location: from adding antibiotics in the form of disks on the top agar layer, to mixing them with the bottom agar. This observation made it possible to plan the first dissertation studies, the aim of which was to determine the most effective modification of the double-layer agar method, allowing the detection of phage-antibiotic combinations with synergistic potential [**D-1**]. The lytic T4-like bacteriophage and 43 different antibiotics belonging to various classes were used in the study. Seven different modifications of the DLA method were tested in terms of the antibiotic application placement and the presence or absence of bottom agar. The studies showed that the total number of phage plaques on the plates depended mainly on the antibiotic used. Differences in the number of plaques depended on the type of modification of the DLA method. It was proved that in order to best visualize the PAS effect, the best results were obtained by the modification with the use of antibiotic disks, in which there was a general increase in the diversity of phage plaque diameters as a result of the application of the antibiotic directly to the top agar layer in the presence of the bottom agar, which could be primarily due to slow diffusion of the antibiotic to the bacterial growth zone. However, the highest total number of plaques was obtained by adding the antibiotic to the bottom agar with the presence of the top agar. This indicates that although the antibiotic could show the PAS effect by the standard disk method, it would be worth investigating whether the effect is equally satisfactory when applying antibiotics directly to the bottom agar, in relation to the use of the same bacteriophage and bacterial host [**D-1**].

Scientific research dealing with the topic of nanomaterials and bacteriophages mainly analyzed the possibility and applicability of creating nanomaterials from components or even entire phage structures. Due to the lack of literature describing the interaction of bacteriophages and nanomaterials (treating nanomaterials as a second, autonomous antimicrobial agent), it was decided to conduct innovative research aimed at experimentally determining their co-application capabilities as part of this dissertation [**D-2**]. The lytic bacteriophage T4-like and six different nanomaterials in the form of nanoparticles (NP) were used in this work: SiO_2, TiO_2-SiO_2, TiO_2, Fe_3O_4, Fe_3O_4-SiO_2 and SiO_2-Fe_3O_4-TiO_2. In the paper it was examined in detail: the plaque-forming ability of the phage, phage lytic efficiency, the time of phage progeny formation and their titers based on the determination of their eclipse phases. Transmission electron microscopy (TEM) imaging and nanoparticle zeta potential (ZP) results were shown to be crucial to explaining the obtained microbiological data. During the interpretation of the results, a hypothesis was proposed that the mere presence of the nanoparticle charge is not sufficient for the bacteriophage to adhere in an orderly and specific way to the nanoparticles, consequently affecting the performance of the phage. The zeta potentials of the nanoparticles had the greatest impact on the observed interactions. ZP thresholds were set at: $ZP < -35$ (mV) for phage tail binding effect and $ZP > 35$ (mV) for phage head binding effect. When the nanoparticles did not meet these requirements, the physical interactions of the phage-nanoparticle mixes became non-specific. It was also shown that the nanoparticles affected the lytic activity of the phage, regardless of their concentration used. Most of the studied nanoparticles had a positive effect on the lytic efficiency of the phage, with the exception of SiO_2 and Fe_3O_4-SiO_2, with the ZP potential lower than 35 (mV), binding to the phage tail [**D-2**].

The antibacterial effect of plant extracts is a subject of unflagging interest from the scientific community, often described in the professional literature. However, papers describing the effect of extracts on lytic bacteriophages is a relatively rare topic, and available publications are largely outdated, so they also use very basic and inaccurate methods. Studies presenting the results of combining plant extracts and phages in a bacterial environment are even rarer, which is why it was decided to conduct interaction analyzes of these multifactor mixtures using many modern research methods [**D-3, unpublished paper B-1**]. Reference lytic bacteriophages (MS2, T4 and phi6) and methanolic plant extracts obtained from echinacea (*Echinacea purpurea*) and rue (*Ruta graveolens*) were used in this study. Due to the complexity of interactions, the following experiments were carried out: evaluation of the effect of plant extracts on bacterial host cells using the microdilution method, evaluation of the effect of plant extracts on the activity and plaque-forming ability of phages by co-incubation method, synographies of static interactions of plant extracts and phages in the bacterial host environment, phage lysis profiles during dynamic growth experiments of the tested mixtures in bioreactors and visualization of the effect of plant extracts and phages on bacterial host cells using a scanning electron microscope (SEM). The studies showed the presence of antagonistic interactions: extracts of echinacea and rue showed antiphage and bactericidal activity. It was proved that the effect of low extract concentrations on microorganisms depended on the tested phage and bacterial hosts species, while high concentrations generally inhibited bacterial lysis. Moreover, the interactions observed in the static environment differed from those performed in the dynamic environment, which indicated the crucial importance of performing multiple analyzes when studying such complex mixtures [**D-3**].

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