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PROSPECTS OF BACTERIOPHAGES IN MANAGEMENT OF PLANT PATHOGENIC BACTERIA

Background. *In recent years bacteriophages draw attention as environmentally friendly means for controlling the bacterial infection. Their use is an effective measure for number of bacterial crops diseases. Bacteriophages have a number of advantages over other methods of control: they are highly specific to host bacteria, non-toxic to macroorganisms, not pathogenic for normal biota of plants and soil. In particular, phage cocktails with specificity and effectiveness against only certain groups of bacteria are used for preventing the spread of infectious diseases in plants. Therefore, the main objective of the study was to isolate and identify bacteriophages specific to phytopathogenic bacteria.*

Methods. *Samples of beet, potatoe, apple, garlic and mandarin with rotting symptoms were selected from vegetable storehouses. Phages were isolated by direct isolation. Phage titers were determined as plaque forming units (pfu/ml) using the standard double layer agar assay technique. The morphological features of viral particles were examined by electron microscopy.*

Results. *As a result of this work we isolated phages (7591/3, 8573/3, Ser/2) specific to Pseudomonas syringae lachrymans 7591, P. fluorescens 8573 and Serratia marcescens. The phage isolates differed in terms of the morphology of their negative colonies. To identify host range of isolated phages spectrum of lytic activity was studied against 16 laboratory strains of phytopathogenic bacteria. Our research revealed that among 9 phage samples three phage isolates are polyvalent.*

Conclusions. *The obtained data indicate that isolates with a broad spectrum of lytic activity can be promising biological agents in control of bacteriosis. Consequently, isolated polyvalent bacteriophages can be considered for therapeutic use against bacterial infection in plants during storage.*

Keywords: *plant pathogenic bacteria, bacterial cultures, morphological features, bacteriophages.*

Background

Human population has been steadily increasing over the last few centuries. In order to meet the global food demand of the growing world's population, there is a need to increase the production of agricultural products. In addition, it is necessary to reduce crop losses due to bacterial diseases. Despite the existing available methods of treating bacterial diseases, there is a need to develop new environmentally friendly strategies to combat them. Current control methods using antibiotics are losing their effectiveness due to the natural development of bacterial resistance to these agents (Anaghez, 2022; Buttimer, 2017). On the other hand, there are no effective traditional antimicrobial drugs against some of agricultural crops diseases (potato blackleg and soft rot disease caused by members of *Enterobacteriaceae*) (Buttimer, 2017; Czajkowski, 2011). That is why agents such as bacteriophages belonged to environmentally friendly approaches of bacterial diseases control have received increased research interest in recent years (Holtappels, 2019; Nawaz, 2023; Wójcicki, 2023).

It is difficult to imagine natural biocenoses without bacteriophages, which participate in the regulation of microbial populations, affect their enzymatic and synthesizing activity (Drulis-Kawa, 2015). Local viral populations are formed under natural conditions. Artificial introduction of bacteriophages into the natural environment is great deal of attention, allowing to detect and predict their impact in more detail. The importance of this task is due, in particular, to the influence of technogenic factors that can accelerate the evolution of populations of phages and bacteria, the divergence of their characteristics and lead to changes in the structure of ecosystems.

In recent years, agriculture has increasingly faced bacterial diseases that are less sensitive to the existing chemical methods of control. In turn, increased concentrations of such chemical substances can lead to damage to plant tissues as reflected in higher crop losses. A major disadvantage of this method of control of phytopathogenic bacteria is also the chemical contamination of crops. Control of bacterial diseases is problematic due to the lack of effective bactericides, high variability of pathogens, high population growth under optimal conditions, development of pathogen resistance to drugs, etc. Bacteriophage-based drugs are considered safe for the environment and human health. Of particular interest are bacteriophages with polyvalent properties, i.e., capable of infecting (and lysing) several different bacteria (García, 2023).

There are two types of bacteriophages relative to the host – specific, i.e., those infecting a limited number of strains of one type of bacteria, and polyvalent ones. Polyvalent phages are able to infect not only certain strains of one species of bacteria, but also different species and/or genera and groups of bacteria (Sovinska, Korotyeyeva, & Andriychuk, 2017; Sovinska, Petrenko, & Andriychuk, 2015). This ability of certain bacteriophages can be the key in combating dangerous bacterial pathogens in various areas of our lives, including medicine, agriculture and the food industry (Drulis-Kawa, Majkowska-Skrobek, Maciejewska, 2015; Halawa, 2023; Faidiuk, 2015). Their practical significance for biological control of microbial populations is undoubtable. Despite the practical and fundamental importance of such phages, bacteriophages

with a wide host range are rarely described (Loc-Carrillo, & Abedon, 2011). One of the reasons is that a sufficiently reliable method of isolation and a model for studying such viruses have not been developed yet. Because the primary population of any phage isolated from a particular medium is considered heterogeneous, that is, in addition to the main phage, there will be other 'contaminating' phages, as well as mutant forms.

The main objective: to investigate the biological properties of isolated bacteriophages to verify the prospects of their use for antibacterial therapy of plant pathogens.

Methods

Samples of plants (beet, potatoe, apple, garlic and mandarin) with symptoms of bacterial lesions were investigated. In our research we used test bacteria: *Pseudomonas syringae lachrymans* 7591, *P. fluoresc*ens 8573 and *Serratia marcescens*. Phages were isolated by direct isolation. Phage titers were determined as plaque forming units (pfu/ml) using the standard double layer agar assay technique (Mohammadali, & Nilsson, 2015; Sovinska, Korotyeyeva, & Andriychuk, 2017). Pure bacteriophage lines were acquired by 6-times passaging with subsequent accumulation on sensitive bacteria cultivated in commercial nutritional broth with additional aeration at 25 ºC. The morphological features of viral particles were studied using electron microscope JEOL-1400 (Japan) (Holtappels, 2021; Mohammadali, & Nilsson, 2015).

Results

In this research samples of beet, potatoe, apple, garlic and mandarin with rotting symptoms were selected from vegetable storehouses. The main symptoms of bacterial lesions observed in fruits were areas of mild decay, softening of the tissues, and the extraction of the exudate. After selection of vegetable samples with symptoms of bacterial lesions bacteriophages specific to phytopathogenic bacteria were obtained. As a result of this work we isolated phages (7591/3, 8573/3, Ser/2) specific to *Pseudomonas syringae lachrymans* 7591, *P. fluorescens* 8573 and *Serratia marcescens*. All selected phages reproduced with the same efficiency on bacteria strains used in this study.

Bacteriophages led to the formation of negative colonies with large (5 ± 1 mm) and medium (2 ± 0,1 mm) sizes depending on the test bacterium (fig. 1).

Fig. 1. Phage plaque morphology on test culture
a – *Serratia marcescens*, b – *P. fluorescens* 8573,
c – *Pseudomonas syringae lachrymans 7591* (plaques diameter = 5 ± 1 (a); 2 ± 0,1(b); 2 ± 0,1 (c) mm)

Isolates 7591/3, affecting *Pseudomonas syringae lachrymans* 7591; 8573/3 of *P. fluorescens* 8573; and an isolate Ser/2 of *Serratia marcescens* were selected among all the isolated bacteriophgages. These isolates were chosen due to the stability of their biological activity during storage, the formation of transparent lytic zone, which allows them to be considered as virulent (non-lysogenic) to indicator strains of phytopathogenic bacteria used in this study. The latter condition is mandatory for the practical use of phages as antagonists of phytopathogenic microbiota in ecosystems, as lysogenization of natural strains by phages introduced for therapeutic purposes does not reduce the number of bacteria, but only provides their immunity.

To identify host range specificity of isolated phages, their spectrum of lytic activity was studied against 16 strains of phytopathogenic bacteria. Our research revealed that from 9 phage isolates three expressed lytic activity against different strains of phytopathogenic bacteria. Hence, these phages possessed a wide range of lytic activity (i.e., these were polyvalent bacteriophages) and could be used as perspective biologic agents for controlling bacterial diseases (fig. 2).

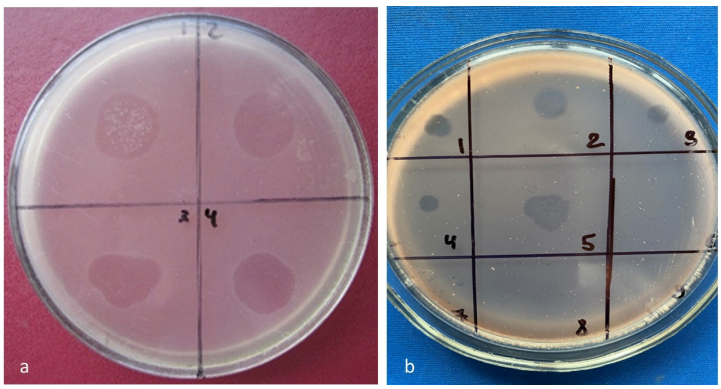

Fig. 2. The spectrum of phages' lytic activity against test bacteria:
a – *Pseudomonas fluorescens 8573,* b – *Serratia marcescens*

Analysis of electron microscopic observations showed that investigated bacteriophages had different morphology and size. Phage particles were icosahedral structures with a head of approximately 43 ± 1 nm in diameter and a short tail of 1 ± 0,5 nm in length, visually belonging to the C1 morphotype of *Podoviridae* family, *Caudovirales*. Transmission electron microscopy also showed the presence of another group of bacteriophages with morphology typical for representatives of *Siphoviridae* family of *Caudovirales* order (icosahedral head with long tail, size – head diameter 67 ± 2 nm, tail length 120 ± 3 nm) (fig. 3).

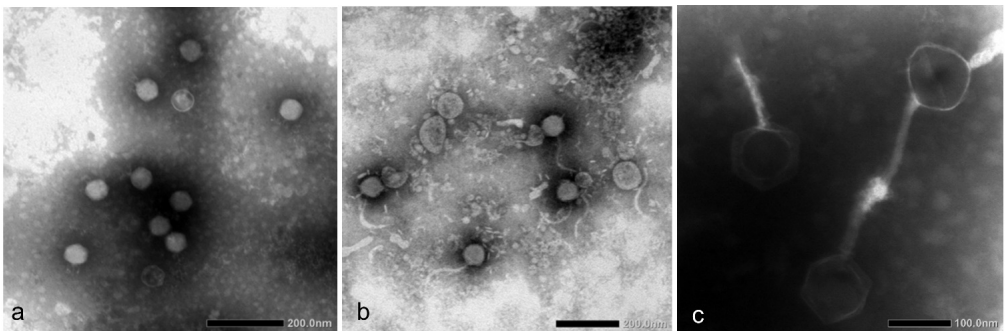

Fig. 3. Electron microscopy images of phage isolates: virions of phages belonging to the *Podoviridae* (a) and *Siphoviridae* (b, c) families

Discussion and conclusions

Plant diseases caused by bacteria are a serious problem in the cultivation and storage of agricultural products. Among such pathogens the most important are bacteria related to the *Pseudomonas* and *Serratia* genera (Sovinska, Petrenko, & Andriychuk, 2015). Using such species as test bacteria, the selection of specific phages is carried out. The selected phages possessed a wide range of lytic activity against these bacterial species and virtually eliminate them. That is why one of the alternative approaches for controlling bacterial diseases is the application of bacteriophages as natural bacterial antagonists (Clokie, 2011; Tahir, 2022). Due to the high specificity of phages against host bacteria, such therapy will destroy the pathogenic biota without affecting the normal one. In addition, the ability of phages to self-replicate allows to use low doses of phage preparations to protect plants. Thus, the use of phages led to a reduction of peach morbidity caused by *Xanthomonas pruni* at 86 – 100 %. In the US, a patent was granted for the production of phage-based antimicrobials to control legume infections caused by *Pseudomonas syringae* (Ramos, 2012).

In this study using selected phages, we have composed a biological plant protection product for use in agriculture. This product has a selective effect on phytopathogenic bacteria, doesn't pollute the environment, increases the profitability of production and provides environmentally friendly agricultural production. Its application could reduce the exposure of toxic chemicals on food products. Biologically active preparation was designed for protection against bacterial infections of sugar beet plants and reduction of rot during the storage of agricultural products. The active ingredients of the preparation were naturally occurring bacteriophages that selectively and specifically killed only certain strains of microorganisms.

On the basis of specific bacterial viruses (bacteriophages) a biologically active preparation for protection against bacterial infections of plants is developed. Product is provided an all-natural, nontoxic, safe and effective means for significantly reducing or eliminating disease-causing bacteria. The laboratory conducts preliminary tests of the drug for controlling plant bacteriosis in agriculture.

Authors' contribution: Olena Andriichuk designed the study; Olena Andriichuk, Hanna Korotieieva and Iryna Budzanivska prepared material for the experiments and collected data; Olena Andriichuk, Hanna Korotieieva and Iryna Budzanivska performed the experiments; Olena Andriichuk and Hanna Korotieieva wrote the paper. All authors discussed the results, commented and approved on the manuscript.

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ПЕРСПЕКТИВИ ВИКОРИСТАННЯ БАКТЕРІОФАГІВ У БОРОТЬБІ З ФІТОПАТОГЕННИМИ БАКТЕРІЯМИ

В с т у п . ***Бактеріофаги останнім часом викликають підвищений дослідницький інтерес як екологічно чисті засоби протидії бактеріальним інфекціям. Їх застосування є ефективним заходом боротьби проти бактеріальних захворювань сільськогосподарських культур. Бактеріофаги мають низку переваг порівняно з іншими методами контролю збудників бактеріозів: вони високоспецифічні до бактерій-хазяїв, не токсичні для макроорганізмів, не шкодочинні для нормобіоти рослин і ґрунту. Зокрема, використовують фагові коктейлі, які виявляють специфічність та уражують лише певні групи бактерій, запобігаючи розповсюдженню інфекційних захворювань у рослинах. Отже, метою дослідження було виділення та ідентифікація бактеріофагів, специфічних для фітопатогенних бактерій.***

М е т о д и . ***З овочесховищ було відібрано зразки буряка, картоплі, яблуні, часнику й мандарина з ознаками гнилі. Фаги виявляли шляхом прямого висіву. Титри визначали у бляшкоутворювальних одиницях в 1 мл (БУО/мл) методом двошарового агару за Граціа. Морфологію віріонів вивчали методом трансмісивної електронної мікроскопії.***

Р е з у л ь т а т и . ***Виділено фаги (7591/3, 8573/3, Ser/2), специфічні до Pseudomonas syringae lachrymans 7591, P. fluorescens 8573 і Serratia marcescens. Морфологія негативних колоній ізолятів фагів відрізнялася. Досліджено спектр біологічної активності щодо 16 штамів фітопатогенних бактерій. Серед досліджуваних дев'яти зразків фагів три виявилися полівалентними.***

В и с н о в к и . ***Отримані дані свідчать про можливість використання ізолятів із широким спектром літичної дії як перспективних біологічних засобів у боротьбі з бактеріозом. Отже, ізольовані полівалентні бактеріофаги можна розглядати для терапевтичного використання проти бактеріальної інфекції при зберіганні рослинної продукції.***

К л ю ч о в і с л о в а : ***фітопатогенні бактерії, бактеріальні культури, морфологічні особливості, бактеріофаги.***

Автори заявляють про відсутність конфлікту інтересів. Спонсори не брали участі в розробленні дослідження; у зборі, аналізі чи інтерпретації даних; у написанні рукопису; в рішенні про публікацію результатів.

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; in the decision to publish the results.