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PROSPECTIVE USE OF BACTERIOPHAGES AS AGENTS TO COMBAT PHYTOPATHOGENIC BACTERIA OF POTATOES

SUMMARY

Plant diseases caused by bacteria are a serious problem in the cultivation and storage of agricultural products. During the last years bacteriophages have attracted increased research interest as a realistic environmentally friendly means for controlling bacterial diseases. The main objective of the study was to characterize the bacteriophages that could be used in the search for and developing the antimicrobial agents based on bacterial viruses. Four isolates of bacteriophages specific to bacteria from *Pseudomonas* genus were isolated from potato samples with typical symptoms of bacterial disease. These phage isolates differed in terms of the morphology of their negative colonies. Slight variation in the morphology of studied phages was observed using electron microscopy. A group of phages was identified as Podoviridae family of Caudovirales order (icosahedral head without long tail, small size – head diameter 43 ± 1 nm, tail length $1 \pm 0,5$ nm). To identify host range/specificity of isolated phages, we analyzed the spectrum of lytic activity against 15 strains of phytopathogenic bacteria. Our research revealed that among four phage samples three expressed lytic activity against different strains of phytopathogenic bacteria. The aforementioned data enable us to conclude that these three isolates with broad spectrum of lytic activity can be used as perspective biologic agents in control of bacteriosis. Thereby phages from our collection could be of therapeutic interest, they have the potential to be used in future prospects for phage therapy research.

Keywords: bacteriophage, bacteriosis, biocontrol, potato.

INTRODUCTION

Last decade witnessed more frequent agricultural challenges connected with bacterial diseases exhibiting resistance to chemical agents of control (Campbell, 2006; Hajek, 2004; Rombouts et al., 2016; Jones et al., 2007; Kabeil et al., 2008). Besides, increasing concentration of these compounds may cause damage to plant tissues and lead to more great lesions of plant crops. Significant disadvantage of currently available antimicrobial methods for combating phytopathogenic bacteria is also chemical contamination of crops (Buttimer et al., 2017). Study of bacteriophages aims at their active practical use in agribusiness.

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Paper presented at the 8th International Scientific Agricultural Symposium "AGROSYM 2017".

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Bacteriophages have received growing interest for agricultural industry as a realistic environmentally friendly means of controlling bacterial diseases, especially in case of the emergence of resistant pathogens. In addition, study of phages reveals the mechanisms of regulating the number and structure of bacterial populations in different environments allowing improve and fine-tune the strategies used for phage therapy (Grey et al., 2001; Born et al., 2011; Birck et al., 2006). At the same time, the search for lytic bacteriophages with a wide range of hosts (covering not only one type of bacteria strains but different species and even genera of bacteria) is the primary purpose for the creation of high therapeutic phage commercial mixes (Bae et al., 2012).

The main objective of the study was to characterize the bacteriophages with practical potential for the search and developing the antimicrobial agents based on bacterial viruses.

MATERIAL AND METHODS

Samples of potato variety Slavyanka and beet variety Mulatka from Kiev and Chernihiv region with symptoms of bacterial lesions were collected in 2014-2015. In our research we used test bacteria: *Pseudomonas syringae* pv. 223 tabaci, *Pseudomonas savastanoi* pv. phaseolicola 4013, *Pseudomonas syringae* pv. lachrymans 7591 and *Pseudomonas fluorescens* 8573. Phages were isolated by direct seeding. 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°C. The morphological features of viral particles were studied using electron microscope JEOL-1400 (Japan) (Faidiuk et al., 2015).

RESULTS AND DISCUSSION

Biodiversity of bacteriophages specific to bacterial diseases and development a collection of phages as agents of phage therapy were the areas of research. During the study four isolates of bacteriophages (223, 7591, 4013, and 8573) have been isolated and accumulated from potato samples with signs of bacterial rot. To identify the host range of selected isolates, the spectrum of phages' lytic activity was determined against 15 strains of pathogenic bacteria. Four isolates of selected phages were shown to exhibit lytic activity against different strains and species of pathogenic bacteria. Our research revealed that three (223, 7591, and 8573) out of four phage isolates expressed lytic activity against *Pseudomonas syringae* pv. 223 tabaci, *Pseudomonas syringae* pv. lachrymans 7591 and *Pseudomonas fluorescens* 8573. However, 4013 isolate expressed lytic activity against host bacteria *Pseudomonas savastanoi* pv. phaseolicola 4013 and against *P. syringae* pv. lachrymans.

It shows that the selected phages possessed a wide range of lytic activity (i.e., polyvalent bacteriophages) and could be used as perspective biologic agents in control of bacterial diseases. Electron microscopy analysis showed that selected phages (223, 7591, 4013, and 8573) differed in colony morphology, virions' size was typical for representatives of *Podoviridae* family of

Caudovirales order (icosahedral head without long tail, small size – head diameter 43 ± 1 nm, tail length $1 \pm 0,5$ nm) (Fig.1).

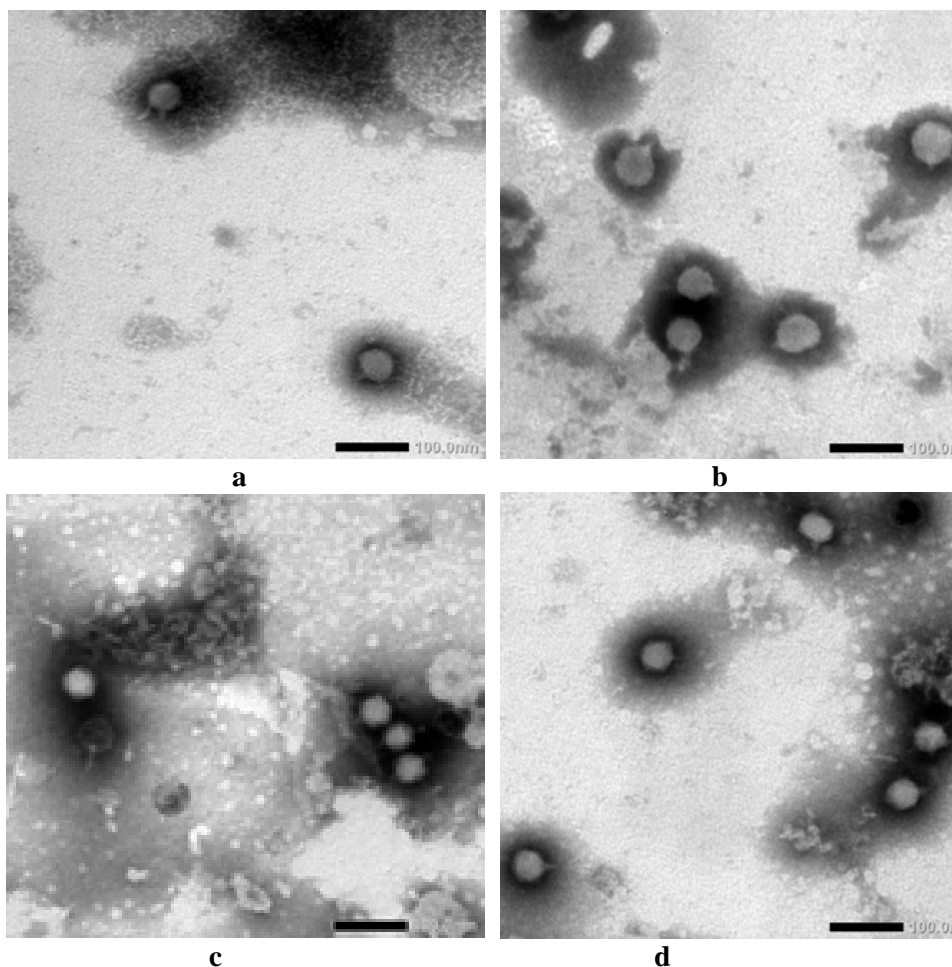


Figure 1. Electron omicroscopy images of phage isolates: a) 223; b) 7591; c) 4013; d) 8573

Practical bacteriophages use to control plant pathogenic bacteria is safe for the environment and human health. Thus, development of an effective preparation based on lytic bacteriophages is the key to successful strategy for control of bacterial plant diseases. There are just a few papers describing use of *Pseudomonas*-specific phages in agriculture. However, representatives of this genus are able to affect a wide range of crops and have serious impact not only on the process of plant cultivation but also the preservation of plant produce during storage. Therefore, we have studied the efficiency of lysis of bacterial population in several samples of important agricultural crops, such as potato and beet.

Samples of potato and beet were treated with a mixture of bacteria *Pseudomonas syringae* pv. 223 tabaci, *Pseudomonas savastanoi* pv. phaseolicola 4013, *Pseudomonas syringae* pv. lachrymans 7591 and *Pseudomonas fluorescens* 8573. The first signs of bacterial lesions on the sections developed by the third day, and the maceration of almost all observed sections has occurred by the fifth day (Sovinska et al., 2015).

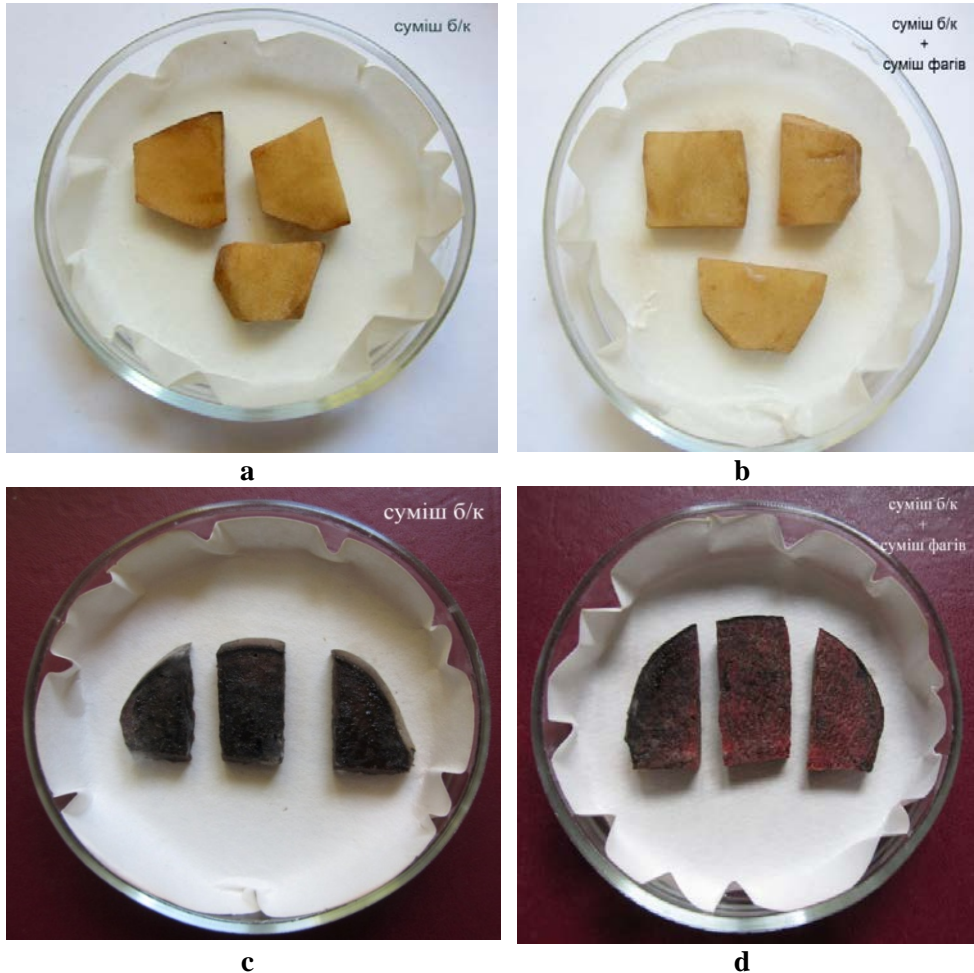


Figure 2. Effectiveness of bacteriophages against bacterial cultures on potato and beet on Petri dish. Plant samples on “a” and “c” were inoculated with bacteria only while plant samples on “b” and “d” were inoculated with bacteria and subsequently with bacteriophage.

There were no active maceration of plant tissue in samples of potatoes and beet post fifth day of simultaneous application of bacterial mixture (*Pseudomonas syringae* pv. tabaci 223, *Pseudomonas savastanoi* pv. phaseolicola 4013, *Pseudomonas syringae* pv. lachrymans 7591 and *Pseudomonas fluorescens* 8573) and a mixture of four isolates of bacteriophages

(phages 223, 4013, 7591, and 8573). This indicates that tested bacteriophages efficiently lysed the bacterial cells. Further, each separate isolate of bacteriophages was tested on sensitive bacteria. No visible destruction of plant tissue was observed in three to five days' time after treatment of samples with a mixture (bacteria + phage), contrary to samples treated with bacteria only. Data on bacteriophage morphology can be used for the development of pathogenic bacteria biocontrol methods in the environment.

The study presents an effective method of using bacteriophages, which is important for laboratory practice and for creation a commercial bacteriophage mixes for controlling plant pathogenic bacteria. Polyvalent phages can be used in search and for creating antimicrobial agents based on bacterial viruses (Sadunishvili, 2013; Żaczek et al., 2015). The isolated phages may be considered as an effective component in the creation of therapeutic phage mixtures (Rene et al., 2008).

CONCLUSIONS

The resulting collection of phages can be used for the development of antimicrobial agents based on bacterial viruses in agriculture. Use of lytic enzymes of isolated phages for controlling bacterial growth is another perspective approach.

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