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THE INFLUENCE OF UV PROTECTANTS ON THE BACTERIOPHAGE ABILITY TO SUPPRESS FIRE BLIGHT DEVELOPMENT IN THE PLANTS TISSUES

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Abstract*. Bacteriophages are considered to play an important role in the fire blight management as the natural habitants of the pome orchards and highly specific bacterial parasites. The main problem of the phage therapy application against fire blight is the necessity to suppress the pathogen development in the phyllosphere were they are easily inactivated by unfavorable environmental factors such as changes of temperature, pH and sunlight irradiation. UV irradiation is critical for bacteriophages and serious efforts are being made for to find effective and economically acceptable UV protectants of the bacterial viruses' particles. In this study it has been shown that casein, soy peptone and carrot juice protected bacteriophages from the UV irradiation damage and phages were able to suppress development of the fire blight causative agent in plant tissues.*

Keywords: bacteriophages, Erwinia amylovora, phage therapy, UV protectants, UV radiation.

INTRODUCTION

Microbial pathogens damage approximately a quarter of the world's plant crops [1]. The causative agent of fire blight disease, bacteria *Erwinia amylovora*, is among the pathogens, responsible for the pome cultures crops losses. In 2012 it was included in the Top 10 bacterial plant pathogen list as voted for by plant bacteriologists associated with the journal Molecular Plant Pathology [2]. Bacteriophages (phages), i.e. bacterial viruses are considered to play an important role in the fire blight management as the natural habitants of the pome orchards and highly specific bacterial parasites. The main problem of the phage therapy application against fire blight is the necessity to suppress the pathogen development in the aerial parts of the plants. It means that the disease control agents have to be able to survive in the phyllosphere which permanently exists under the pressure of such unfavorable factors as changes of temperature, pH and sunlight irradiation, especially of its UV spectrum.It has been demonstrated that bacteriophages are easily inactivated by all factors mentioned above [3].

By now there are very few commercially available preparations on the basis

of bacteriophages against the most phyllosphere bacterial pathogens, including *Erwinia amylovora,* due to the high vulnerability of the phage particles to the aggressive abiotic factors. Since UV irradiation is critical for bacteriophage application in the plants disease control, serious efforts are being made for to find effective and economically acceptable UV protectants of the bacterial viruses' particles. Substances of different nature, able to minimize the negative effect of UV rays were tested in laboratory and field conditions [4, 5, 6]. Natural, organic compounds such as lignin, black and green tea, coffee and cocoa extracts, have shown high level of the protection of the virus particles from the UV- induced damage [7].

The aim of this work was to study the effect of several UV protectants on the ability of bacteriophages to suppress development of bacteria *E. amylovora* in immature pome fruits under the controlled conditions.

MATERIALS AND METHODS

Bacteria *Erwinia amylovora* were cultivated on the LB agar (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, 20 g/L agar) at 28˚C. Bacteriophages *E. amylovora* were cultivated by a standard double agar overlay method on the LB agar (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, 20 g/L agar) and soft LB agar (0.7% agar). Liquid cultures were grown in LB medium (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl) at 28˚C. Immature pear fruits were inoculated with suspension of *E. amylovora* EaM contained 10^4 CFU/ml and treated with 10^7 PFU/ml of bacteriophage isolates with casein, soy peptone and carrot juice, prepared as described by Born et al [4].

Immature pear fruits infected with bacteria and treated with bacteriophages with different UV protectants were exposed to UV light, wavelength 254 nm, for 1, 3 and 5 minutes. Immature pear fruits, treated with sterile distilled water and immature pears, infected with bacteria *E. amylovora* and treated with bacteriophage lysates without UV protectants were used as the controls. The experimental pear fruits were incubated in the wet chambers at the temperature +28˚C. Symptoms of the disease were recorded at 1, 2, 3, 4 and 7 days after inoculation. For each bacterium/phage with UV protectants' combination tested pears were assayed in triplicate and each experiment was repeated at least two times. The assessment of the degree of *E. amylovora* infection on the immature pear fruits was carried out according to the following scale: 0 – absence of the necrosis; 1– necrotic spots on the 5% of the fruit's surface; 2– necrotic spots on the 25% of the fruit's surface; 3 – necrotic spots on the 50% of the fruit's surface; 4 – necrotic spots on the 75-100% of the fruit's surface and/or ooze formation.

RESULTS AND DISCUSSIONS

In the natural conditions all participants of the system "plant - pathogen - biocontrol agent" are under the influence of the environmental biotic and abiotic factors. In the pome orchards plants affected with bacteria, fire blight causative agent bacteria *Erwinia amylovora*, and bacterial viruses are UV irradiated at the same time. Experiments, carried out in the controlled conditions can present information concerning the ability of the UV protectants to provide such protection of bacteriophages that they were able to suppress development of the pathogen in the plant tissues.

Three substances: casein, soy peptone and carrot juice, known for their ability to absorb UV waves [4, 5, 6] were used in the assays (Figure 1.).

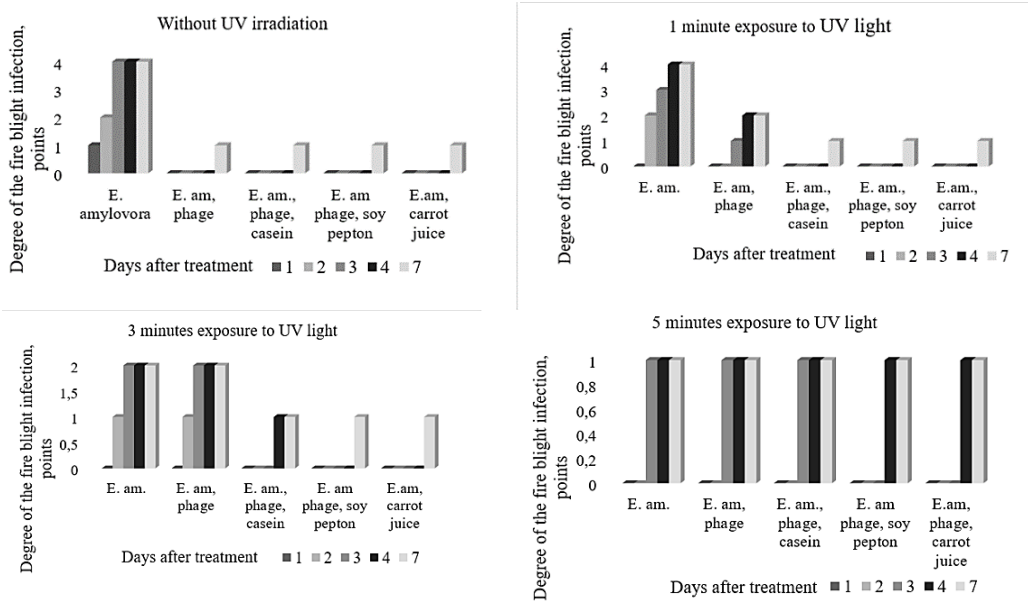


Figure1. Degree of *Erwinia amylovora* infection on the immature pear fruits treated with phage lysates with UV protectants and exposed to UV light.

In the absence of UV irradiation phage lysates without UV protectants and with UV protectants successfully destroyed bacterial cells in the pear tissues. On the fourth day of experiment, when pear fruits, infected with pathogenic bacteria, produced typical milky white ooze, there were single necrotic spots on the bacteriophages treated pear fruits in all variants with phage lysates treatment.

As expected, with increased exposure to UV light the amount of bacteria and bacteriophages decreased. After one-minute exposure to UV light the first necrotic spots on the immature pear fruits infected with bacteria appeared in 24

hours after infection. Bacterial population, exposed to UV light for 1 minutes, recovered virulence on the fourth day of the experiment. On the pear fruits, infected with bacteria and treated with phage lysate without UV protectant 50% of the surface was covered with necrotic spots. At the same time, no more than 5% of the pear fruits surface, infected with bacteria and treated with phage lysates contained UV protectants, were covered with necrotic spots.

Three minutes' exposure to UV light partially destroyed bacterial cells, so that on the fourth day of the experiment the 50% of the immature pear fruits surface, infected with bacteria *Erwinia amylovora,* were covered with necrotic spots. The same degree of the pathogen infection was revealed on the pear fruits, treated with phage lysate without UV protectants. Pear fruits, infected with bacteria and treated with bacteriophage lysates contained UV protectants, demonstrated 5% necrosis on their surface.

After five minutes' exposure to UV light the single necrotic spots on the immature pear fruits were recorded in all variants. It can be assumed that in this case just a single bacterial cells were alive and bacteriophages in all phage lysates were inactivated.

In this study the substances, which absorb UV light due to the presence of aromatic amino acids - casein, aromatic amino acids tryptophan, tyrosine, and phenylalanine within its peptide structure – soy peptone, carotenoids – carrot juice were able to provide protection of the bacteriophage particles in the plants tissues at least for 96 hours after three minutes' exposure to UV light. Phage lysates without UV protectants suppressed the development of the pathogen in the plant tissues at the same level as phage lysates with UV protectants when they were not irradiated by UV rays. There were no significant differences in the action durability of the UV protectants used in the experiment.

The control efficacy of bacteriophage-based treatment in agriculture can be influenced by numerous biotic and abiotic factors as well as by the way of treatment such as soil drenching, foliar spraying, infiltration etc. [8]. Besides, bacteriophages can be destroyed by chemical preparations used for the pests control. However, bacteriophages advantages as biological control agents are worthy of all these limitations being overcome. Phage formulations for commercial purposes should be effective, economically attractive and easy to use. That is why it is important to select the most effective phage-based preparations for the target bacterial pathogen.

CONCLUSIONS

The selection of the most biological and cost effective UV protectant of the bacterial virus's particles is necessary for to use the high potential of phage therapy. The results of the experiments have shown that tested UV protectants casein, soy peptone and carrot juice demonstrated ability to protect phage particles from UV

light. Phage particles, treated with the studied UV protectants kept the degree of the *Erwinia amylovora* infection on the immature pear fruits on the low level after exposure to UV light.

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