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BACTERIOPHAGES IN THE QUINCE TREES PROTECTION AGAINST THE FIRE BLIGHT DISEASE

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Summary. The system "bacteria *Erwinia amylovora* — bacteriophages *Erwinia amylovora*" has been investigated in the quince shoots under the controlled temperature and humidity and the absence of the most inhabitants of the orchard microbiocenosis; in the quince plants under the partially controlled conditions of the experimental plot and in the commercial quince orchard under the pressure of the biotic and abiotic stressors. In all three investigated environments *E. amylovora* bacteriophages, used in the experiments, demonstrated ability to suppress the fire blight pathogen bacterium *Erwinia amylovora* growth in the quince plants tissues.

Keywords: *biological control, bacteriophages, bacteriophage therapy, fire blight, Erwinia amylovora, quince.*

Introduction. Fire blight, caused by the bacterial phyto-pathogen *Erwinia amylovora,* is one of the economically most important diseases of fruit trees. Plants are extremely vulnerable for fire blight infection at the bloom stage. Blossom blight can lead to the great crop losses and even the plant death. Currently available bacteriosis control agents as, e.g., copper preparations or antibiotics act mostly unspecifically and are prone to resistance development. Alternative disease management strategies are, therefore, highly solicited.

Bacteriophages, i.e. highly specific bacterial viruses that infect and lyse bacteria, are under intensive evaluation as the biocontrol agents (Sabri et al., 2022, Kim et al., 2022). The advantage of phages is that they are natural components of ecosystems, infect only bacteria sensitive to them and are non-toxic to plants, animals and humans. Bacteriophages are widely spread and can be detected in the targeted bacteria natural environment. It is important that, bacteriophages can regulate themselves in the sites of infection, depending of the bacterial population density. Being highly specialized bacterial parasites bacteriophages can be applied

against antibiotic resistant pathogenic bacteria or when chemical control measures are forbidden, for example, during plants bloom stage.

Recent studied have demonstrated that under the conditions of the natural biocenosises microorganisms use mechanisms which are not visible under the controlled conditions. For instance, the genes which participate in the pathogen's infection process with low expression levels in the *E. amylovora* inoculum growth in LB medium and increase their expression level at some point during flower infection were detected just in the last years (Schachterle et al., 2022).

Furthermore, it is already clear that there are social interactions, including cooperation, altruism, and cheating among and between bacterial hosts and bacteriophages which infect them. Understanding the sociobiology of bacteriophages will have implications for the therapeutic use of bacteriophages to treat bacterial infections (Secor and Dandekar, 2020).

Thus, the aim of the present research was to study the interaction of the system "bacteria *Erwinia amylovora* – bacteriophages *Erwinia amylovora*" under the different environments in order to understand how to create the most effective means of protection against fire blight on the basis of bacteriophages.

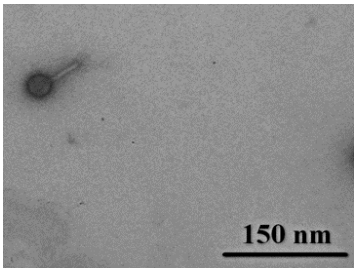
Materials and methods. Bacteria *Erwinia amylovora* were cultivated on the LB agar (1*0* g/L *peptone, 5* g/L *yeast extract, 10* g/L *NaCl, 20 g/L agar*) at 28˚C. Bacteriophages *E. amylovora* were cultivated by a standard double agar overlay method on the LB agar (1*0* g/L *peptone, 5* g/L *yeast extract, 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.

Quince shoots in the controlled conditions were investigated in the following variants: infected with bacteria *E. amylovora,* infected with bacteria *E. amylovora* and treated by bacteriophages *E. amylovora,* treated by water. In the conditions of the experimental plot quince trees were infected with bacteria *E. amylovora,* infected with bacteria *E. amylovora* and treated by bacteriophages *Erwinia amylovora,* treated by water and infected with *E. amylovora* and treated by preparation on the basis of copper. In the commercial quince orchard, the quince plants were treated by bacteriophages *E. amylovora,* treated by preparation on the basis of copper and there were a few non-treated trees.

The disease prevalence was calculated as follows:

$P = n / N \times 100\%$, where n – number of affected plants; N – total number of plants sampled.

Results and discussions. Plants and shoots in the experiment were treated with the *Erwinia amylovora* bacteriophages, which we had previously isolated from quince plants affected by fire blight (Figure 1).



A

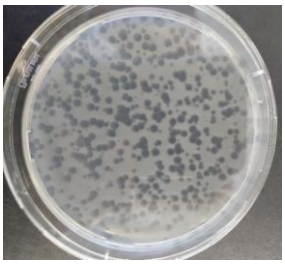

B

Fig. 1. Bacteriophages used in the study: A - phage particles under the electron mycroscope,
B- double-layer assay showing the phage plaques forming units

For the infection of the experimental plants the *E. amylovora* bacterial suspension in concentration 10^3 CFU/ml was used. The concentration of phages suspension was 10^8 PFU/ml. Experiment on the quince shoots was conducted under the temperature +28˚C and 95% humidity. Action of bacteriophages on the development of the causative agent of fire blight on the quince plants at the experimental plot was carried out at the medium temperature +17.7˚C and relative humidity 31% which were during the critical experimental period. In the commercial quince orchard treatments with bacteriophages were carried out during full quince blossoming, critical for fire blight infection and when any treatments with pesticides are forbidden, at the medium temperature +25˚C and relative humidity 43%. Results of the interaction of the system "bacteria *Erwinia amylovora* — bacteriophages *Erwinia amylovora*" in different environments are presented in the table 1.

Table 1. Bacteriophages *E. amylovora* impact on the fire blight disease prevalence in the different environments.

Variant	Disease prevalence, %			
	Bacteria *E. amylovora*	Phages + bacteria *E. amylovora*	Water	Copper preparation
Quince shoots, controlled conditions	97%	18%	-	

Quince plants, experimental plot	67%	7,14%	25%	67%
Quince plants, commercial orchard	-	32%	52%	32%

It is evident that in all variants bacteriophages suppressed the pathogenic bacteria *E. amylovora* in the experimental plant tissues. The best effect in suppression of fire blight pathogen development was observed under conditions of temperature and humidity favourable for its development and in the absence of the most members of the quince orchard microbiocenosis. The lowest suppressive effect, which was nevertheless at the level of the protective action of copper-containing preparations, was observed in a commercial quince orchard. Namely, in conditions where bacteriophages are exposed to the UV radiation, high temperatures, low humidity, and strong pressure of the orchard microbiocenosis inhabitants. The conducted experiments have shown that bacteriophages *E. amylovora* which we isolated and characterized, have the inhibitory effect on the causative agent of fire blight, bacterium *E. amylovora* not only under conditions favourable for the development of the system "bacteria *Erwinia amylovora* — bacteriophages *Erwinia amylovora*", but also under strong environmental pressure.

Conclusions. It is shown that bacteriophages effectively inhibit the development of the fire blight causative agent in host plant tissues under optimal conditions for the development of the "bacteria *Erwinia amylovora* — bacteriophages *Erwinia amylovora*" system. At the same time, at the high environmental pressure on phages, their effectiveness, although reduced, remains at the level provided by chemical means of protection against this bacteriosis. The obtained results confirm the effectiveness of bacteriophages in the fire blight control and emphasize the necessity of differentiated approach to the application of bacteriophages in plant protection against this dangerous disease of the fruit trees.

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