

## **ABSTRACT**

dissertation for the degree of Doctor of Philosophy (Ph.D.) in the specialty  
"6D070100 – Biotechnology"

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on the thesis: "Development of a method for use of microorganisms against the  
causative agent of fire blight in apple orchards of Kazakhstan"

### **General description of work.**

The dissertation work is devoted to screening and studying the inhibitory activity of collection and new strains of microorganisms isolated from host plants and developing a method for their use against the causative agent of fire blight (*Erwinia amylovora*) of fruit crops in Kazakhstan.

### **Relevance of the research topic.**

In recent years, a serious problem has arisen in the fruit growing of Kazakhstan caused by a disease such as fire blight, which is considered one of the most dangerous diseases that have quarantine status. In terms of the degree of harmfulness, none of the known diseases of fruit trees can be compared with it. As a result of damage by this disease, young seedlings of fruit trees can die in one season, old orchards - in 2-3 years. Economic damage is expressed in a decrease or complete loss of harvest, costs of uprooting dead trees and planting a new orchard. Pears are mainly susceptible to this disease, but the disease can also affect apple trees, hawthorn, quince, and rowan. Unfortunately, the scale of the disease is expanding every year. It can damage all fruit trees and shrubs of the *Rosaceae* family.

At the moment, there are no effective measures against the causative agent of fire blight *E. amylovora*. Chemical control methods are used only to reduce the development of infection and prevent new infections. Modern fungicides, except those containing copper, do not act on the causative agent of fire blight. In the literature regarding measures to combat fire blight, it is proposed to spray trees with 0.5-1% Bordeaux mixture or 0.3% copper oxychloride at the beginning of budding, before and immediately after flowering, as well as after harvesting. Spraying during flowering is effective, while it is noted that regular spraying of copper-containing preparations in gardens has led to mutations in the bacterium *E. amylovora*, as a result of which its varieties have appeared that are resistant to this method of protection. An alternative and most effective way to reduce the pathogen population is the use of biologics. The main advantage of this method is that it does not have a harmful effect on the environment and its biota. This is due to the fact that active antagonistic strains are isolated from the same epiphytic environment. Therefore, in recent years, throughout the world, including in our country, much attention has been paid to the search and development of technology for producing biologics based on active microorganisms that can inhibit the growth of the causative agent of fire blight.

In this regard, the development of a method for using microorganisms that have inhibitory activity against the causative agent of fire blight in apple orchards of Kazakhstan is very relevant.

**The purpose of research:** Determination of the effectiveness of microorganisms with inhibitory activity against the causative agent of fire blight of fruit crops and development of a method for their use.

**Research objectives:**

- Conducting phytosanitary monitoring of fruit (apple) orchards in various ecological and geographical regions of Kazakhstan and identifying new strains of microorganisms.
- Screening and selection of collection strains of microorganisms and newly isolated isolates with antagonistic activity against *E. amylovora*.
- Study of the influence of ultraviolet rays on the inhibitory activity of selected strains against the causative agent of fire blight of fruit crops.
- Identification of the main metabolites in the culture liquid of selected strains and determination of their inhibitory activity against *E. amylovora*.
- Development of a method for using selected strains and determining their biological effectiveness against the causative agent of fire blight.

**Objects of research.**

The work used collection cultures of microorganisms from the Research and Production Center for Microbiology and Virology, as well as new isolates isolated from samples (fruits, leaves, branches) of the apple and pear phyllosphere collected during route surveys in the industrial horticulture zones of Zhambyl, Turkestan and Almaty regions of Kazakhstan. The studies also used the Polish isolate 48M, isolated from apple leaves at the Department of Phytopathology, The National Institute of Horticultural Research (Skierniewice, Poland).

**Research methods.**

During the research work, generally accepted phytopathological, microbiological, biochemical, molecular-genetics and chemical research methods were used.

**Scientific novelty of the research.**

The essence of the novelty of the research results is isolate of new and selection of active strains of epiphytic microorganisms that exhibit antagonistic activity against the causative agent of fire blight, common in the orchards of Kazakhstan.

For the first time in Kazakhstan, microorganisms with antagonistic activity were selected and proposed to combat bacterial blight of fruit crops.

For the first time, bacteria such as *Bacillus amyloliquefaciens*, *Lactobacillus plantarum* and a collection strain of actinomycete *Streptomyces canofumeus*, which have high inhibitory activity against the causative agent of fire blight *E. amylovora*, were isolated and studied.

The main metabolites in strains that showed antagonistic activity against *E. amylovora* were determined.

As a result of irradiation of the bacterium *B. amyloliquefaciens* with ultraviolet rays, a mutant strain MB40 with high productive properties was obtained.

A method for using strains *B. amyloliquefaciens* MB40 and *L. plantarum* 17M against the causative agent of fire blight of fruit crops has been developed and their effectiveness has been assessed.

### **Theoretical significance and practical value of research.**

As a result of the screening, highly active strains of microorganisms were selected, the main metabolites that determine their antagonistic activity were identified, and methods for using the selected strains against the causative agent of fire blight of fruit crops in Kazakhstan were developed.

The results obtained will make it possible to use isolated active strains of microorganisms with antagonistic activity to create domestic biological products against the causative agent of fire blight (*E. amylovora*) of fruit crops. The results of the research work will contribute to the reduction or elimination of the use of chemicals, as well as the biological protection of fruit trees from fire blight, which will lead to an increase in the quality and improvement of life of the population by providing it with environmentally friendly products.

It should be noted that preparations based on bacterial cultures will be more cost-effective, much cheaper, since they do not require additional costs for isolating antibiotics; their preparative form will be a cultural liquid containing spores, bacterial cells and biologically active metabolites.

Thus, this dissertation work on the development of a method for using microorganisms with antagonistic activity against the causative agent of fire blight of fruit crops has great theoretical significance and practical value.

### **The main provisions submitted for defense:**

1. Screening of collection and new strains of microorganisms allows us to identify highly active strains that suppress the growth of the causative agent of bacterial blight of fruit crops *E. amylovora*.

2. Irradiation with ultraviolet rays makes it possible to obtain a mutant strain of *B. amyloliquefaciens* MB40, which is 2.8 times more active than the original parent strain.

3. The study of the component composition of the culture liquid of promising strains made it possible to identify the main metabolites and determine their inhibitory activity against the causative agent of bacterial blight.

4. The developed method of using experimental samples of biologics based on antagonistic bacteria (*B. amyloliquefaciens* MB40 and *L. plantarum* 17M) makes it possible to effectively protect fruit trees from fire blight.

### **The main research results and conclusions:**

1. During the phytosanitary monitoring of garden cenoses in Zhambyl, Turkestan and Almaty regions of Kazakhstan, 216 isolates of the following taxonomic groups were isolated from the collected 47 samples (fruits, leaves, branches) of the apple and pear phyllosphere: bacteria – 142, fungi - 37 and yeast - 37 isolates.

2. The screening of collection and new strains of microorganisms showed that the greatest antagonistic activity against the causative agent of fire blight *E. amylovora* was shown by *L. casei* 139, *L. casei* 173, *St. canofumeus* K20/1, *B.*

*amyloliquefaciens* MB40, *L. plantarum* 17M and the Polish strain *P. agglomerans* 48M.

3. As a result of irradiation with ultraviolet rays, a mutant strain of *B. amyloliquefaciens* MB40 was obtained, exceeding the activity of the original strain by 2.8 times.

4. The main metabolites in the culture fluid of the selected strains were identified, causing inhibitory activity against *E. amylovora*. The strain *B. amyloliquefaciens* MB40 has acetoin and 2,3-butanedione, as well as the lactic acid strain *L. plantarum* 17M has 2,3-butanedione, acetic and lactic acids.

5. A method has been developed for using prototypes of biologics based on strains *B. amyloliquefaciens* MB40 and *L. plantarum* 17M against fire blight of fruit crops, which consists of twice treating garden trees with a cultural liquid at a consumption rate of 4 l/ha, which allows effectively protecting fruit trees from fire blight. On the susceptible apple variety "Aport", the biological effectiveness of experimental samples of biologics based on strains *B. amyloliquefaciens* MB40 and *L. plantarum* 17M was  $70 \pm 0.2\%$  and  $83.4 \pm 0.4\%$ , respectively, on the resistant variety "Star Crimson" -  $75.3 \pm 0.5\%$  and  $89 \pm 0.1\%$ , respectively.

#### **Personal contribution of the author.**

The author independently carried out an analysis of the literature data on the research topic, experimental work, statistical processing, and analysis of the research results and preparation of the dissertation manuscript.

#### **Connection with the plan of the main scientific work.**

Scientific research of the dissertation work was carried out within the framework of grant funding of the research project and the program: AR05131543 "Development of a biologics against the causative agent of fire blight of fruit crops" and BR18574022 "Microbial preparations for combating fire blight of fruit crops". Scientific supervisor, Doctor of Biological Sciences, Professor A.K. Sadanov.

#### **Approbation of work.**

The main provisions of the dissertation work were reported and discussed at International conferences, including:

- International scientific conference of students and young scientists "Farabi Alemi" within the framework of the V International Farabi Readings (Almaty, 2018);
- International Symposium: "Astana Biotech 2018" (Astana, 2018);
- International Conference Conservation Asia: "Actualizing Conservation in a Changing Asia" (Bishkek, 2018);
- 20th International Conference on Biodiversity and Ecosystems (Tokyo, 2018);
- 4th International Symposium on the biological control of bacterial plant diseases (Viterbo, 2019);
- 2nd International Symposium on Fire Blight of Rosaceae (Michigan, 2019)
- XX EURO Analysis (Istanbul, 2019);
- International scientific and practical conference Innovations in agriculture and ecology (Ryazan, 2020);

- 12th International Congress on Plant Pathology (Lyon, S.63, S.67, 2023);
- International scientific and practical conference dedicated to the 65th anniversary of the Scientific Research Institute of Biological Safety Problems of the Ministry of Health of the Republic of Kazakhstan (Almaty, pp. 268-269, 2023).

### **Publications.**

28 works have been published in the open press based on the dissertation materials, including 4 articles in foreign scientific journals, indexed in the *Web of Science* or *Scopus* databases with a non-zero impact factor (European journal of plant pathology 2020- 70% (Q2), Journal of plant pathology 2020 – 41% (Q3), World journal of microbiology and biotechnology 2023 – 73% (Q2); Journal of plant pathology 2024 – 56% (Q2)); 5 articles in republican scientific journals recommended by the Committee for Control in the Sphere of Education and Science of the Republic of Kazakhstan; 4 articles in domestic rating publications, 1 article and 11 abstracts in materials of international conferences. Based on the results of the work done, 2 patents of the Republic of Kazakhstan for an invention and 1 application were received, with a decision to issue a patent for the invention: 1) No. 34036, registration No. 2018/0536.1 dated November 29, 2019; 2) No. 35238, registration No. 2020/0149.1 dated 09/03/2021; 3) registration No. 2023/0167.1 dated 03/07/2023.

**The scope and structure of the dissertation.** The dissertation work is presented on 120 pages of computer text and consists of the following sections: normative references, definitions, notations and abbreviations, introduction, literature review, materials and research methods, results and their discussion, conclusion, list of used sources of 245 titles. The work includes 18 tables, 41 figures and 3 appendices.