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In the specialty 8D05105 - Biotechnology.

Thesis for the degree of Doctor of Philosophy (Ph.D)

ABSTRACT

on the topic: **«Development of a microbial preparation to combat bacterial burn of fruit crops»**

General characteristics of the work. The dissertation is devoted to the development of a biological preparation against bacterial burn of fruit crops caused by the pathogenic bacterium *E. amylovora* E22. In the course of monitoring studies, the area of this disease in garden cenoses of Kazakhstan was determined. A strain of lactic acid bacterium *Lacticaseibacillus paracasei M12* with high antagonistic activity against the pathogen of bacterial burn has been isolated. The component composition of biologically active substances produced by this microorganism has been determined. Based on the strain *L. paracasei M12*, the biological preparation "Lactin AS" was developed, which was tested on various varieties and ecological zones of gardening in Kazakhstan. Its biological effectiveness against the pathogen *E. amylovora* E22 was 90.7%. The biopreparation "Lactin AS" significantly reduces the development of bacterial burns and is an alternative to existing pesticides.

Relevance of the research topic. Bacterial burn caused by the phytopathogenic bacterium *E. amylovora* E22 poses a serious threat to fruit plantations, especially in regions with favorable agroclimatic conditions, such as the southern and southeastern regions of Kazakhstan. The pathogen affects a wide range of seed crops, including apple (*Malus domestica*), pear (*Pyrus communis*) and a number of other fruit plants, causing tissue necrosis, drying of shoots and tree death. The disease is characterized by a high rate of spread and difficulty in control, which causes significant economic losses. The consequences of infection include both direct losses from loss of harvest and indirect costs associated with sanitary measures, including the removal of infected trees, soil disinfection and restoration of fruit agrocenoses.

Currently, there are no effective measures against the bacterial burn agent *E. amylovora* E22. Scientifically recommended measures to combat this disease are pruning the affected branches or digging up trees with roots, removing them and treating them with copper-containing preparations and antibiotics. Chemical control methods are used to reduce the progression of infection and prevent new infections. Modern bactericides, except for copper-containing ones, do not act on the burn pathogen. Unfortunately, currently in the republic there is not a single domestic drug to combat bacterial burn of fruit crops. Therefore, the creation and production of a domestic biological product against the burn pathogen will reduce dependence on imported supplies of drugs. The use of biological products to combat the pathogen *E. amylovora* E22 is the most promising direction in the development of methods for obtaining environmentally friendly products, which is of social and economic importance for the development of agriculture.

Purpose of the work: To develop a microbial biopreparation against the causative agent of bacterial burn of fruit crops *E. amylovora* E22 and determine its biological effectiveness.

Tasks of the research work:

1. Monitoring of fruit crops in South Kazakhstan for the presence of bacterial burns and screening of microorganisms with inhibitory activity.
2. Analysis of the metabolic composition of the culture liquid of the strain, which has active antagonistic properties, and molecular and genetic identification of microorganisms.

3. Study of phytotoxicity of the biopreparation "Lactin AS", created on the basis of the strain *Lacticaseibacillus paracasei* M12.

4. Study of the influence of the microbial preparation "Lactin AS" on the biochemical metabolites of fruit crops.

5. Development of a microbial drug against bacterial burns based on a promising antagonistic strain and determination of its biological effectiveness.

Objects of research: Apple trees of varieties *"Zarja Alatau", "Aport", "Star Krimson",* as well as pear varieties of the variety *"Shygys"* were considered as fruit crops.

The causative agent of bacterial burn *E. amylovora* E22 was isolated from the exudate of the damaged apple tree fruit of the "*Aport*" variety, collected in the peasant economy of the Karasai district of Almaty region.

Strains of microorganisms, fungi and actinomycetes were obtained from the collection of the Research and Production Center of Microbiology and Virology LLP.

The strain *Lacticaseibacillus paracasei* M12 was isolated from the phyllosphere of the orchard in Almaty region.

Research methods. During the study, classical phytopathological, microbiological, biochemical, molecular-genetic, as well as physicochemical methods of analysis were used.

Scientific novelty of research work. The scientific novelty of the dissertation is that on the basis of the active antagonistic strain of the lactic acid bacterium *Lacticaseibacillus paracasei* M12, adapted to the natural and climatic conditions of Kazakhstan, an original microbial preparation directed against the causative agent of bacterial burn of fruit crops was developed for the first time in the country.

Theoretical significance and practical value of the study. Bacterial burn is one of the most dangerous diseases of fruit crops. The creation of a biological preparation based on antagonist microorganisms against bacterial burns of fruit crops will significantly reduce dependence on imported supplies of drugs. Biopreparation "Lactin AS" is an excellent alternative to chemical preparations for plant protection. It contains biologically active metabolites and is absolutely safe for humans.

This drug has a number of advantages:

1. The biological product is non-toxic to humans and animals, which makes it safe for use in household and gardening.

2. Ease of use: preparation of the working solution with the biological preparation "Lactin AS" does not require special skills and is available even to inexperienced gardeners. In addition, the consumption of the biological preparation for the preparation of the solution is much less compared to chemicals.

3. The biological effectiveness of the biological preparation "Lactin AS" against the pathogen of bacterial burn was 90.7%. After spraying, fruit crops become immune to the causative agent of bacterial burn, which ensures their long-term protection. These characteristics only make the biological product "Lactin AS" effective.

The main provisions recommended for protection:

1. Against the pathogen E. amylovora E22, strains were selected that restrain the growth of bacterial burns of fruit crops and showed high antagonistic activity - from the soil 2 strains (16.2, 19.2) and 1 strain of M12, selected from the phyllosphere of cenoses of garden plants.

2. In the component composition of the culture fluid of the strain *L. paracasei* M12, three main components have been identified that have a bactericidal effect against the causative agent of bacterial burn - acetic acid, lactic acid and 2,3-butandione.

3. The drug "Lactin AC" is developed on the basis of the strain *L. paracasei* M12, which allows you to protect fruit trees from bacterial burns with an efficiency of 90.7% when used twice in the phases "before flowering" and "after full flowering".

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Main results and results of scientific work

1. During the monitoring of fruit crops in the farms of the South Kazakhstan region (Almaty, Zhambyl and Turkestan regions), outbreaks of bacterial burns caused by *E. amylovora* E22 were detected, not only on apple and pear, but also on plums, cherries, quice, peaches and wild stone rocks. The spread and development of the disease manifested themselves to varying degrees depending on the growing area, varietal characteristics of plants and weather conditions. As a result of the screening of the antagonistic activity of microorganisms, three strains with high inhibitory activity against *E. amylovora* E22, isolated from the affected fruits of the Aport apple tree variety (Karasai district, Almaty region) were isolated. Isolates 16.2 and 19.2, obtained from the soil of Turkestan and Zhambyl regions, respectively, demonstrated pathogen growth inhibition zones of 52.2 ± 0.5 mm and 45.6 ± 0.5 mm. The M12 isolate, isolated from the phyllosphere of the garden cenosis of the Almaty region, provided a suppression zone of 41.5 ± 0.5 mm. Molecular genetic identification showed that the isolate M12 belongs to the species *Lacticaseibacillus paracasei*, while isolates 16.2 and 19.2 are attributed to the genus *Pseudomonas sp*.

2. The antagonistic properties of the *L. paracasei* M12 strain are due to the production of organic acids and volatile metabolites: acetic acid (48.7%), lactic acid (4.8%) and 2,3-butandione (4.2%). These compounds have a pronounced antimicrobial effect and are considered as the main factors that inhibit the growth of *E. amylovora* E22.

3. The phytotoxicity of the biological product "Lactin AS", developed on the basis of the strain *L. paracasei* M12, was evaluated on wheat and cucumber crops. The results showed that the concentrations of the drug in the range from 0.1% to 0.5% contributed to the improvement of wheat growth rates, while concentrations from 0.05% to 0.5% were optimal for cucumber. At 100% concentration of the drug, suppression of the growth of both cultures was observed. Thus, low and medium concentrations of the biological product contribute to the activation of plant growth, while high concentrations have a phytotoxic effect.

4. Physiological and biochemical analysis of plants treated with the drug showed a significant increase in the activity of antioxidant enzymes - catalase (CAT) and peroxidase (POA), especially in pear leaves. At the same time, there was a decrease in the activity of ascorbate oxidase (APO) and cyclooxygenase (COD), which indicates the restructuring of the antioxidant system of

plants and the activation of protective mechanisms under stress conditions. The biological preparation also contributed to an increase in the content of photosynthetic pigments (chlorophyl a, b and carotenoids) in apple leaves, especially in the KT10 variety, which indicates an improvement in photosynthetic activity and increased resistance of plants to stress. The maximum physiological effect was observed on the 30th day after treatment, mainly in wild varieties; by 60 days, the activity decreased, which may indicate the exhaustion of the drug's action.

5. In model and production experiments on testing the biological preparation in garden cenoses, the biological efficiency of the biological preparation "Lactin AS" at a consumption rate of 5 l/ha with two-fold (1st - "butonization" phase, 2nd - "full flowering") processing on a stable apple variety "Starcrimson" amounted to - 90.7%. The "Standard of Organization" and "Technological Instruction" for the production of the biological preparation "Lactin AS" based on the strain of lactic acid bacteria *L. paracasei* M12, intended against bacterial burns of fruit crops, have been developed.

Approval, approval of the results of the work and personal contribution of the author. The materials received during the dissertation work were published in 12 scientific papers, including 3 articles in peer-reviewed foreign scientific publications; 2 articles in international peer-reviewed scientific journals indexed in Web of Science or Scopus databases and included in the Q2 quartile with a non-zero impact factor; 1 article in international peer-reviewed scientific journals included in the Q1 quartile; 6 articles in republican scientific publications recommended by the Committee for Control in Education and Science of the Republic of Kazakhstan; 5 abstracts published in the proceedings of international conferences. According to the results of the dissertation work, the invention passed the state examination and an application for a patent of the Republic of Kazakhstan was submitted.

Personal contribution of the dissertation. Independently conducted an analysis of literary data on the research topic, experimental work, statistical processing, analysis of the research results and preparation of the thesis manuscript.

Connection of work with the scientific research program. The dissertation work was carried out within the framework of the program BR 18574022 "Microbial preparations against the causative agent of bacterial burn of fruit and berry crops". Scientific supervisor of the program: Doctor of Biological Sciences, Professor Sadanov A.K.

Structure and volume of the dissertation work. The thesis consists of 116 pages, normative references, designations and abbreviations, introduction, literature review, research objects, materials and methods, research results and their discussion, conclusions, 241 references, 27 tables, 49 figures.