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Original Article

Monitoring studies of the occurrence of fire blight pathogen in Kazakhstan and identification of antagonistic microorganisms suppressing its development

Estudos de monitoramento da ocorrência do patógeno do fogo bacteriano no Cazaquistão e identificação de microrganismos antagônicos que suprimem seu desenvolvimento

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Abstract

The paper presents data on phytosanitary monitoring of garden cenoses for fire blight in the Turkestan, Zhambyl, and Almaty regions of Kazakhstan. The purpose of this study is to assess the phytosanitary situation in various regions of Kazakhstan, determine the extent of fire blight spread, and isolate and identify the fire blight pathogen. During the study, methods such as hypersensitivity, pathogenicity, and fluorescent simplification-based specific hybridization polymerase chain reaction (FLASH-PCR) were used. It was found that in all the surveyed areas, disease foci were identified. For the first time, the fire blight pathogen was detected on fruit crops such as plum, peach, cherry plum, and quince, as well as on wild apricots. 274 plant samples were collected from which microorganisms were isolated. Isolates related to the fire blight pathogen *Erwinia amylovora* were identified by methods of hypersensitivity, pathogenicity, and FLASH-PCR diagnostics. Of the 156 isolates of microorganisms isolated from apple tree plant samples, 21 inhibited the in vitro growth of *E. amylovora* to varying degrees. Isolates 16.2 and 19.2 with maximum antagonistic activity were selected, where the pathogen growth inhibition zones were 52.2 ± 2.58 mm and 45.6 ± 0.55 mm, respectively. Based on the obtained sequence of nucleotides of the 16SpRNA gene site, it was found that the selected isolates with high antagonistic activity belonged to the *Pseudomonas* genus. In the future, based on these isolates, a new biological product for fire blight control can be created and adapted to the natural and climatic conditions of Kazakhstan.

Keywords: fruit crops, monitoring, *Erwinia amylovora*, microbial antagonists, polymerase chain reaction analysis.

Resumo

O presente artigo apresenta dados sobre o monitoramento fitossanitário da biocenose de um jardim em relação à doença do fogo bacteriano nas regiões do Turquestão, Zhambyl e Almaty no Cazaquistão. O objetivo deste estudo é avaliar a situação fitossanitária em diversas regiões do Cazaquistão, além de determinar a extensão da propagação, isolar e identificar o patógeno da doença do fogo bacteriano. Durante este estudo, foram usados métodos como hipersensibilidade, patogenicidade e reação em cadeia da polimerase com hibridização específica baseada em simplificação fluorescente (FLASH-PCR). Foram identificados focos da doença em todas as áreas pesquisadas. Pela primeira vez, o patógeno da doença do fogo bacteriano foi detectado em culturas de frutas como ameixa, pêssego, abrunheiro-de-jardim e marmelo, bem como em damascos silvestres. Foram coletadas 274 amostras de plantas das quais foram isolados os microrganismos. Os isolados relacionados ao patógeno *Erwinia amylovora* foram identificados por métodos de hipersensibilidade, patogenicidade e diagnóstico com FLASH-PCR. Dos 156 microrganismos isolados de amostras de macieira, 21 inibiram o crescimento in vitro de *E. amylovora* em graus variados. Os isolados 16.2 e 19.2 com máxima atividade antagônica foram selecionados, apresentando zonas de inibição do crescimento do patógeno de 52,2 ± 2,58 mm e 45,6 ± 0,55 mm, respectivamente. Com base na sequência obtida de nucleotídeos do local do gene 16SpRNA, verificou-se que os isolados selecionados com alta atividade antagônica pertenciam ao gênero *Pseudomonas*. Com base nos isolados supracitados, um novo produto biológico para o controle da doença do fogo bacteriano poderá ser criado no futuro, estando adaptado às condições naturais e climáticas do Cazaquistão.

Palavras-chave: culturas frutíferas, monitoramento, *Erwinia amylovora*, antagonistas microbianos, análise de reação em cadeia da polimerase.

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1. Introduction

Fire blight *Erwinia amylovora* (Burrill, 1882) affects more than 160 species of plants of the *Rosaceae* family. The disease causes especially serious damage to the plantations of quince, pear, and apple trees (Beikzadeh and Varasteh, 2020; Doukkali et al., 2022; Johnson, 2000). Sour cherry, apricot, rose, juneberry, strawberry, raspberry, plum, and sweet cherry are less susceptible to fire blight. The *E. amylovora* pathogen has the status of a quarantinable disease agent, is found in almost all regions of fruit crop production in the world, and causes great damage (Bonn and van der Zwet, 2000; Erokhova and Orlinskii, 2017). The disease has a wide geographical distribution: cases of *E. amylovora* have been recorded in more than 50 countries around the world (EPPO, 2013). According to van der Zwet (2006), in some countries with cool climates, the spread of fire blight is rarely severe and usually has no economic significance, unlike in warmer and wetter climates. For example, in Southern Europe and the Eastern Mediterranean, the disease occurs from time to time in a very severe form. The presence of fire blight is more likely in the countries located near the disease foci. The harmfulness of fire blight is very high due to its very rapid spread. In heavily infected gardens, it can affect 20-50% of plants, 10-20% of which die completely. In some gardens, where there is no proper care and protective measures are not applied, up to 90% of fruit trees are infected with fire blight. Annual crop losses from fire blight and the cost of fire blight control range from 50 to 100 million dollars (Bonn and van der Zwet, 2000; Norelli et al., 2003; Zhao et al., 2019).

In Kazakhstan, this disease was first discovered in the early 2000s and its range is expanding from year to year. Currently, numerous fire blight foci have been identified in the south and southeast of Kazakhstan, which poses a threat to the Kazakh fruit-growing industry. Now, according to the Statistics Agency of the Republic of Kazakhstan, the area under apple and pear trees is over 36,000 ha. The area infected with fire blight is 419.935 ha, including by regions: in the Almaty region 342.035 ha, the Zhambyl region 72.6 ha, and the Turkestan region 5.3 ha.

The climatic conditions of the south-east of Kazakhstan, where the main fruit growing area is located, according to pest risk analysis (PRA), are favorable for acclimatization and substantiation of the disease pathogen (Drenova et al., 2013; Kairova et al., 2023; Rezzonico, 2014; Zharmukhamedova et al., 2016).

In previously conducted monitoring studies, for the first time in the south and south-east of Kazakhstan and in the adjacent territory of the Republic of Kyrgyzstan, 28 strains of the fire blight pathogen were isolated, identified, and included in the collection of the All-Russian Center for Plant Quarantine (Djaimurzina et al., 2014; Dzhaimurzina et al., 2014; Doolotkeldieva and Bobusheva, 2016). Relatively warm, humid winters with a predominance of positive temperatures, as well as rainy springs with a temperature optimum of +18 °C and high relative humidity, are favorable conditions for the spread of the disease. Besides, dew fall due to a sharp drop in air temperature between night and daytime indicators is a factor of the disease. It was found that bacteria were viable on diseased plants for a long time, resistant to drying and sunlight. In drops of exudate in the light, the disease pathogen persists for 32 hours, in the dark for more than two months, and in dry exudate from 9 months to two years. The pathogen dies when exposed to temperatures in the range of 45-50 °C for 10 minutes (Pusey, 1999). Birds, insects, rain, or wind transmit the disease pathogen. Fire blight spreads with planting and grafting materials, and pruning tools, and is carried by pollinating insects, like bees, wasps, flies, and sucking insects (aphids). The infection can be spread by birds, rain, wind, and irrigation water. There are cases when infected fruits and containers introduce the disease. Over long distances, the fire blight pathogens can be carried mainly by host plants if they have latent infection or undetected lesions.

The development of fire blight symptoms is associated with the seasonal development of the host plant. The flowering period of fruit crops is the phase of greatest danger since mass infection of flowers can occur, leading to an epidemic of fire blight (Thomson, 2000). In this regard, developing measures to localize disease foci without radical destruction of valuable fruit crops is urgent. One of the methods against the fire blight pathogen is the use of biological preparations. This control method is an effective and sustainable alternative or supplement to conventional pesticides for plant disease control. The most intensively studied biological control agents are antagonistic bacteria, which form a natural component of garden microbiocenosis.

The relevance of this promising area of biotechnology is primarily related to the interest shown worldwide in organic farming and the production of environmentally friendly food products. Nowadays, organic farming is practiced in 160 countries around the world (Morgera et al., 2016). Kazakhstan also pays special attention to this field.

Therefore, biological control of fire blight using preparations based on antagonist bacteria, which are natural components of garden microbe biocenoses, can serve as one of the elements of organic farming and be an environmentally friendly alternative to the use of chemical plant protection products (Choi et al., 2022; Mikiciński, 2017; Mikiciński et al., 2022; Pusey et al., 2009; Sobiczewski et al., 2008; Sundin et al., 2009). Currently, the list of pesticides and agrochemicals allowed for use in Kazakhstan includes the only preparation used to treat this disease, i.e., Phytolavin, BRK (a complex of streptotricine antibiotics). Therefore, in Kazakhstan, much attention is paid to the search and isolation of new microorganisms with inhibitory activity against the fire blight pathogen and the development of technologies to produce biological preparations (Sadanov et al., 2023; Shemshura et al., 2020).

Thus, the purpose of our study was to assess the phytosanitary situation in various regions of Kazakhstan and to establish the extent of the progression of fire blight. Furthermore, we wanted to isolate and identify the fire blight pathogen and microorganisms with inhibitory activity from the samples selected during monitoring.

2. Materials and Methods

2.1. Location and time of the study

To detect the disease promptly, the examination was carried out in the spring of 2023 during the period of mass flowering of fruit trees, when flower blight can often be observed. The specified examination period is most favorable for monitoring the manifestation of bacterial diseases in fruit plantations, since outbreaks of bacteriosis fade in summer (Drenova and Artemeva, 2018; Khodzhaeva et al., 2016). From an epidemiological point of view, the first three days after the opening of the flower are the most susceptible to infection.

Therefore, during this period, monitoring surveys of fruit crops growing in the Turkestan, Zhambyl, and Almaty regions of Kazakhstan for blight damage were conducted (Table 1). The survey covered farms where fire blight lesions had previously been found in gardens.

Table 1. Route surveys of cenoses in the Almaty, Zhambyl and Turkestan regions.

Region	Name of the farm	Culture under study
Almaty region	Zhemis farm	Aport apple tree
	Zharyk farm	Golden Delicious apple tree
	O. V. Suzdaleva farm	Maksat apple tree
	Dikhan farm	Starkrimson apple tree
	Alatau farm	Aport apple tree
	Olzhas farm	Aport apple tree
	Issyk State Arboretum Republican State Budget Enterprise (RSBE)	Aport apple tree
	G. Khusainova farm	Golden Delicious apple tree
	Apple World farm	Gala apple tree
	Zhalgasbaev farm	Starkrimson apple tree
	Semirechye farm	Golden Delicious apple tree
	Tan farm	Talgarskaya Krasavitsa pear tree
	Dursunov farm	Starkrimson apple tree
	Turganbaeva farm	Starkrimson apple tree
Zhambyl region	Zhemis farm, Korday	Leto plum tree
	Aruzhan farm, Korday	Golden Delicious apple tree
	Astana farm, Bayzak	Talgarskaya Krasavitsa pear tree
	Altyn Alma Socio-Entrepreneurial Corporation (SEC), Asa	Golden Delicious apple tree
	Sovet farm, Merke	Starkrimson apple tree
	Balabek farm, Merke	Idared apple tree
	Baizhanova farm, Bayzak	Golden Delicious apple tree
	Kargylash farm, Bayzak	Golden Delicious apple tree
	Zhanat, Bayzak	Golden Delicious apple tree
		Quince
	Aisha-Bibi village	Cherry plum
		Talgarskaya Krasavitsa pear tree
	Pan farm, Korday	Cherry plum
	Kungey farm, Korday	Talgarskaya Krasavitsa pear tree
	Maksat-Tere farm	Golden Delicious apple tree
Turkestan region	Amangeldi LLP	Golden Delicious apple tree
	Yntymak Ben Arys farm	Golden Delicious apple tree
	Zhana kush farm	Golden Delicious apple tree
	Alan and Company LLP	Golden Delicious apple tree
		Peachtree
		Talgarskaya Krasavitsa pear tree
	Amangeldy farm	Golden Delicious apple tree
	Birlik SEC	Talgarskaya Krasavitsa pear tree
	Saryagash Zher Syiy LLP	Leto plum tree
		Golden Delicious apple tree
	Maksat Aray LLP	Golden Delicious apple tree
	Alkhory farm	Golden Delicious apple tree
	Kablanbek farm	Golden Delicious apple tree
	An abandoned garden at the exit from Shymkent	Golden Delicious apple tree
	Ak Niet Agro Gardens LLP	Pink Lady apple tree
		Konfetka apple tree
		Idared apple tree
	Keremet-Sapa LLP	Golden Delicious apple tree
	Private plot at the Utepov village	Golden Delicious apple tree

The object of the study was samples of affected plant parts selected during route surveys in industrial gardening zones of the Turkestan, Zhambyl, and Almaty regions of Kazakhstan and cultures of microorganisms isolated from them.

2.2. Monitoring studies of garden cenoses

Route surveys were conducted in the following fruit-growing areas of Kazakhstan: the Almaty region: 14 farms, the Zhambyl region: 13 farms, and the Turkestan region: 14 farms. The assessment of fire blight damage was carried out on zoned pomaceous (apple, pear) and drupaceous (plum, quince, peach, apricot, etc.) crops according to the method described in (Drenova and Artemeva, 2018).

A phytopathological assessment of trees for fire blight damage was carried out on two diagonals of the garden from four sides of the crown. At least 100 trees were examined in each garden. The prevalence of the disease and the degree of its development were determined by special Formulas 1 and 2:

$$P = \frac{a100}{n} \quad (1)$$

where P is the prevalence of the disease (%); *a* is the number of affected leaves or fruits; and *n* is the number of viewed leaves or fruits.

$$R = \frac{n(ab)}{N} \quad (2)$$

where R is the development of the disease (%); (*a b*) is the sum of the products of the number of affected leaves or fruits by the corresponding lesion score; *n* is the total number of viewed leaves or fruits; and *N* is the highest lesion score.

During the examination, samples with typical fire blight symptoms were taken in compliance with all the requirements of antiseptics, 3-4 samples were taken from each diseased tree. During sampling, the affected parts of plants were cut out with the capture of healthy tissue. One sample consists of different plant parts collected from a single tree. Each sample includes inflorescences, seed buds, leaves, fruits, young shoots, bark from parent branches, and the bole. The selected samples were placed in filter paper bags along with a label indicating the region, farm or organization, culture, variety, age, area, and date of sampling. The selected samples in filter bags were stored in the refrigerator for several weeks until the exact etiology of the disease was determined.

2.3. Isolation of microorganisms

The samples collected during the route surveys were rinsed with tap water for 20 minutes. They were then subjected to surface sterilization by immersion in 70% ethyl alcohol for 3 minutes and washed twice with sterile distilled water for several minutes. In the case of flowers or leaves, each sample was macerated in several drops of sterile water in a sterile Petri dish using sterile instruments. Thirty minutes after maceration, 30 µl of macerated tissue was applied in strokes to various nutrient media: sucrose peptone agar (SPA); De Man-Rogosa-Sharpe agar (MRS) or fish visceral protein autolysate (FPA).

The Petri dishes were then incubated at 28 °C for 2-3 days and bacterial growth was checked daily. This procedure was repeated three to four times to ensure that pure cultures were obtained for identification tests.

SPA. 1 litter of SPA medium consisted of K_2HPO_4 (0.5 g), $MgSO_4 \times 7H_2O$ (0.25 g), peptone (5 g), agar (5 g), and sucrose (20 g).

MRS agar (g/l): Proteose peptone: 10, twin-80: 1, glucose (anhydrous): 20, meat extract: 8, yeast extract: 4, D-(+)-glucose: 20, sodium acetate: 5, magnesium sulfate: 0.2, manganese sulfate: 0.05, dipotassium phosphate:2, triamonium citrate: 2, polysorbate 80: 1, agar: 14.

FPA (g/l) fish hydrolyzate: 12, peptone enzymatic: 12, agar: 20.

2.4. Hypersensitivity and pathogenicity test

The hypersensitivity test was performed on the leaves of the *Pelargonium domesticum* L.H. Bailey indicator plant. Suspensions of tested isolates in sterile distilled water (10^7 colony-forming units (CFU)/mL) obtained after washing bacteria from the SPA medium after 24 hours of incubation, were injected into the mesophyll of the *P. domesticum* leaf using a syringe. The reaction was observed after 18-24 hours (Schaad et al., 2001).

The pathogenicity test was performed on unripe pear fruits using a modified technique proposed by Mikiciński et al. (2022). Unripe pear fruits, cut in half, were pre-sterilized with 70% ethanol, then dipped into a test culture broth, placed in sterile Petri dishes on moistened filter paper, and left for 6 hours at 25 °C. Then they were inoculated with a suspension of microbial isolates at a concentration of 10^8 CFU/ml. Symptoms of infection on pears were noted within 7 days after treatment (Mikiciński et al., 2016). The results were evaluated on a 4-point scale given in the work of Kim and Geider (2000) according to which: 0 points mean absence of necrosis and exudate; 1 point means mild necrosis; 2 points mean severe necrosis; 3 points mean necrosis and weak exudate; and 4 points mean abundant exudate and necrosis. The control was pear fruits treated with sterile water (Kim and Geider, 2000).

2.5. Diagnosis of fire blight by fluorescent amplification-based specific hybridization polymerase chain reaction (FLASH-PCR)

Identification of *E. amylovora* in various fruit crop samples selected during monitoring was carried out using reagent kits from AgroDiagnostics LLC (Moscow, Russia) according to the manufacturer's protocol.

Two types of primer pairs were used for PCR amplification: FER1-F: 5'-AGC AGC AAT TAA TGG CAA GTA TAG TCA-3' rgER2-R:5'-AAA AGA GAC ATC TGG ATT CAG ACA AT-3' and PEANT1-F:5'-TAT CCC TAA AAA CCT CAG TGC-3' PEANT2-R: 5'-GCA ACC TTG TGC CCT TTA-3'. When using the FLASH-PCR express method, the detection of the results was carried out on a gene fluorimeter (DNA Technology). At the detection stage, the fluorescence level of the sample is determined with the background

fluorescence level, and the result is given in relative units (as a quotient of the fluorescence value of the sample to the background value). A reaction mixture without polymerase and DNA, but with a PCR buffer, was used as a background. The results are displayed in the form of a table and a diagram (Zavriev et al., 2008).

2.6. Agar diffusion test

For the test, 156 isolates related to the accompanying microflora isolated from apple tree samples were taken. The antagonistic properties of microbial cultures were determined by the presence of a growth inhibition zone when they were co-cultured with a test culture of *E. amylovora* strain 1E IMIV that had been isolated from the affected fruits and shoots of apple growing in the Karasai district of the Almaty region in 2018 (Shemshura et al., 2020) by diffusion into agar (Balouiri et al., 2016). Fermentation of the bacterial culture broth was carried out on MRS media without agar by a deep method on a Biosan incubator shaker with a platform oscillation frequency of 180 rpm and a temperature from 28 to 37 °C, for 2-7 days, depending on the type of microorganism. *E. amylovora* 1E IMIV strain (10^8 CFU/ml) was applied to the surface of the SPA medium and rubbed with a sterile spatula. Then, using a sterile cork drill (D=8 mm), holes were made in this medium. 0.1 mL of culture broth (10^7 CFU/ml) of each tested microorganism was added to the wells. The Petri dishes were then placed in a thermostat for 24 hours at 28 °C, after which growth inhibition zones of the *E. amylovora* 1E IMIV strain were measured around the wells. Sterile water was used as a negative control. After two days of co-cultivation of bacteria, the diameter of the growth inhibition zones was determined (without deducting the diameter of the well) and expressed in millimeters. The test was conducted in two copies, each in five repetitions.

2.7. Molecular genetic characteristics of microbial antagonists

The identification of two bacterial strains was carried out by determining the direct nucleotide sequence of the 16S rRNA fragment of the gene, followed by determining the nucleotide identity with the sequences deposited in the international Gene Bank database, as well as building phylogenetic trees with the nucleotide sequences of the reference strains.

The PCR reaction was performed with universal primers (Vegas et al., 2006) 8f (5'-AgAgTTTgATCCTggCTCAg-3') and 806R (5'-ggACTACCAgggTATCTAAT-3') in a total volume of 30 µl. The PCR mixture contained 25 ng of DNA, 1 unit of Maxima Hot Start Taq DNA Polymerase (Fermentas) 0.2 mM of each dNTP, 1 PCR buffer (Fermentas), 2.5 mM of MgCl2, and 10 pmol of each primer. The PCR amplification program included prolonged denaturation at 95 °C for 3 minutes, in 32 cycles: at 95 °C for 30 seconds, at 55 °C for 40 seconds, at 72 °C for 60 seconds; and final elongation for 10 minutes at 72 °C. The PCR program was performed using the GeneAmp PCR System 9700 amplifier (Applied Biosystems).

2.8. Statistical analysis

The sequencing reaction was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions, followed by fragment separation on an automatic genetic analyzer 3730xl DNA Analyzer (Applied Biosystems). Statistical analyses were carried out using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA).

3. Results and Discussion

It was found that fire blight occurred with varying degrees of damage everywhere. New foci of the disease were discovered. In all surveyed farms in the Turkestan, Zhambyl, and Almaty regions, the P of the disease ranged from 1.5 to 88.0%, with an R from 0.1 to 24.8%, respectively (Figures 1 to 3 and Tables 2 to 4). The maximum manifestation of the disease (P=88.0%, R=24.7%) was observed on a pear of the Talgarskaya Krasavitsa variety in the Tan farm in the Almaty region (Figures 1 to 3 and Tables 2 to 4).

Comparing the manifestation of the disease in the regions of Kazakhstan, we found that the highest indices of blight manifestation on fruit crops were observed in the Zhambyl region (Table 3). The P of fire blight ranged from 20 to 83%, and the degree of manifestation from 1.5 to 4.9%. The lowest rates were recorded in the Turkestan region. The P of the disease in this region ranged from 2.4 to 58%, and the degree of manifestation of the disease was 0.05-14.8% (Table 4).

In farms where all agrotechnical measures are carried out and chemical plant protection products are used against pests carrying the *E. amylovora* pathogen, the spread of the disease was weak or completely absent. In abandoned gardens and in farms where proper protective measures for garden care were not carried out, the spread of the disease on wild apricots was 58.0% with a degree of manifestation up to 14.7% (Figure 4). Individual trees were completely dead.

As a result of the monitoring studies, 274 samples were selected from different regions of Kazakhstan, most of which were apple blossoms. 339 bacterial isolates were isolated from them in the laboratory.

3.1. Hypersensitivity and pathogenicity test

To quickly identify the fire blight pathogen, all isolates were tested for hypersensitivity reactions and pathogenicity using the *P. domesticum* plant. It was found that bacteria similar to *E. amylovora* caused chlorosis with necrosis on the leaves (Figure 5). Non-pathogenic (saprophytic) bacterial species did not cause such a reaction.

Simultaneously with the formulation of the hypersensitivity reaction, a test for the pathogenicity of isolated bacteria on immature pear fruits was performed using a modified White technique (Sobiczewski and Millikan, 1985). As a result of the studies, isolates similar to *E. amylovora* caused tissue necrosis in pear fruits with the release of dirty white exudate (Figure 6). This is one of the main diagnostic signs of *E. amylovora*.

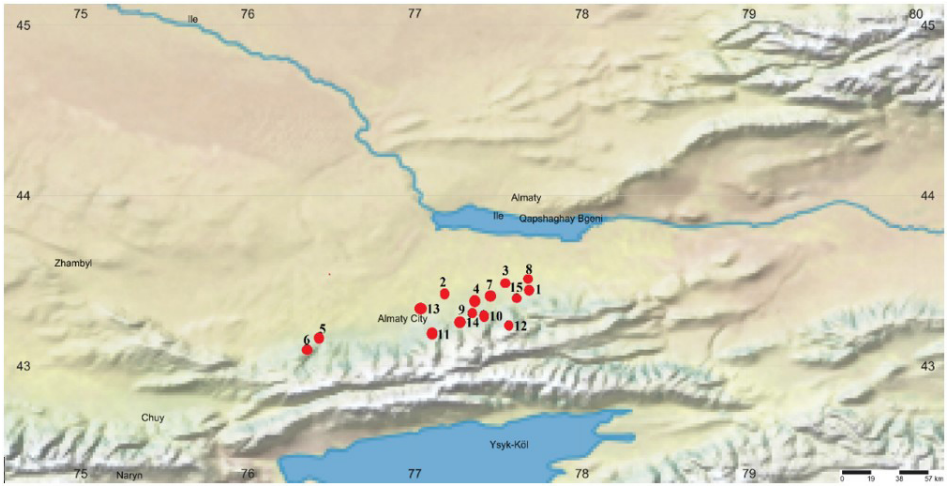

Figure 1. Foci of fire blight in fruit trees in the Almaty region.

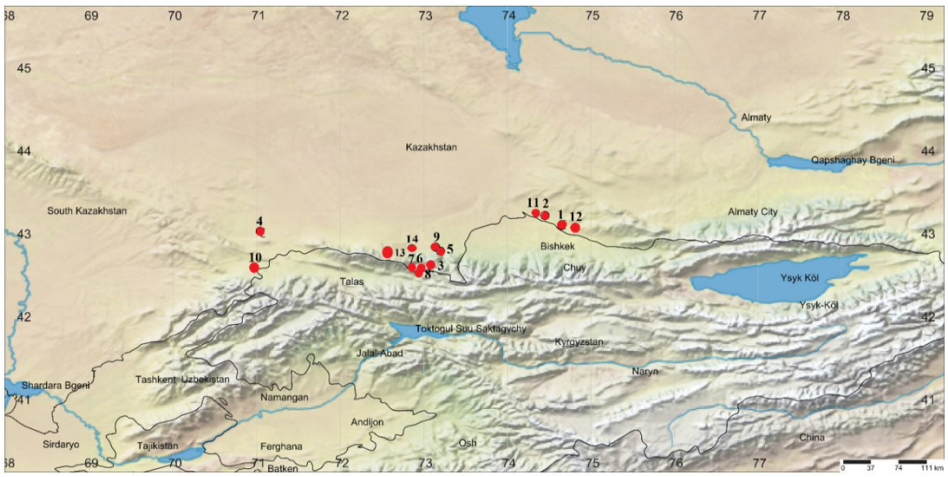

Figure 2. Foci of fire blight in fruit trees in the Zhambyl region.

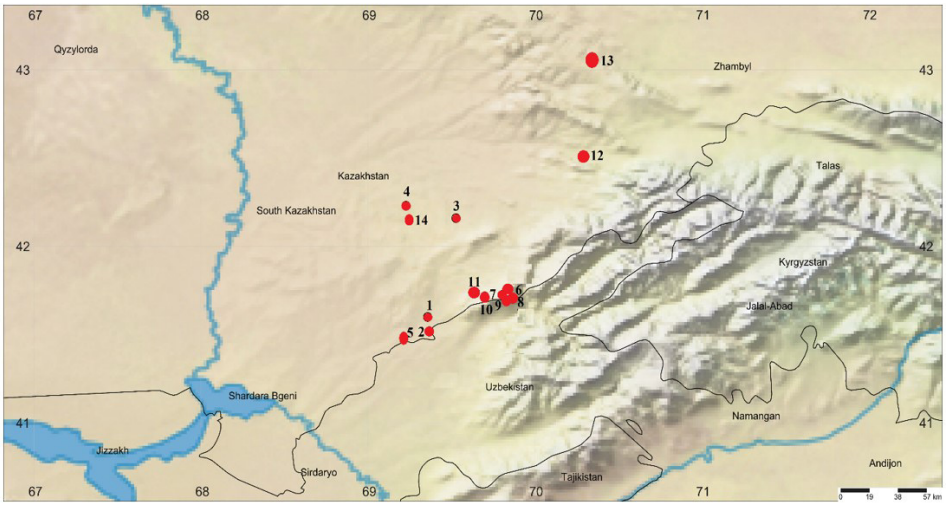

Figure 3. Foci of fire blight in fruit trees in the Turkistan region.

Table 2. Results of phytosanitary monitoring of garden cenoses for fire blight pathogen infection in Almaty region of Kazakhstan, 2023.

No.	Name of the organization or farm	Latitude (N)	Longitude (E)	Disease index, %		Fruit culture, variety
				P	R	
1	Zhemis farm	43º45'88.5"	77º69'64.2"	1.5	0.2	Aport apple tree
2	Zharyk farm	43º43'99.7"	77º18'01.8"	23.3	8.2	Golden Delicious apple tree
3	O.V. Suzdaleva farm	43º49'94.8"	77º55'55"	1.5	0.1	Maksat apple tree
4	Dikhan farm	43º50'75.5"	77º70'03.2"	0	0	Starkrimson apple tree
5	Alatau farm	43°10'44.0"	76°43'43.3"	20.2	4.2	Aport apple tree
6	Olzhas farm	43°09'32.6"	76°33'33.8"	1.3	0.1	Aport apple tree
7	Issyk State Arboretum RSBE	43º45'69.6"	77º45'33.7"	11.5	0.6	Aport apple tree
8	G. Khusainova farm	43°50'84"	77°70'66"	6.2	0.3	Golden Delicious apple tree
9	Apple World farm	43°27'16.3"	77°33'36.3"	10	1.1	Gala apple tree
10	Zhalgasbaev farm	43°31'04.2"	77°44'25.6"	1.6	0.03	Starkrimson apple tree
11	Semirechye farm	43°16'47.6"	77°11'16.8"	0.9	0.02	Golden Delicious apple tree
12	Tan farm	43º18'45.0"	76º63'97.2"	88.0	24.7	Talgarskaya Krasavitsa pear tree
13	Dursunov farm	43º33'12.0"	77º11'09.4"	3.0	0.3	Starkrimson apple tree
14	Turganbaeva farm	43º30'20.1"	77º40'70.7"	2.6	0.4	Starkrimson apple tree

Note: P is the prevalence of the disease; R is the degree of development of the disease.

Table 3. Results of phytosanitary monitoring of garden cenoses for fire blight pathogen infection in the Zhambyl region, 2023.

No.	Name of the organization or farm	Latitude (N)	Longitude (E)	Disease index, %		Fruit culture, variety
				P	R	
1	Zhemis farm, Korday	43º10'54.3"	74º65'73.1"	32.0	1.7	Leto plum tree
2	Aruzhan farm, Korday	43º22'01.5"	74º45'84.9"'	23.0	2.8	Golden Delicious apple tree
3	Astana farm, Bayzak	42º50'74.8"	73º08'54.1"	83.0	4.7	Talgarskaya Krasavitsa pear tree
4	Altyn Alma SEC, Asa	43º03'18.9"	71º04'28.0"	38.0	3.8	Golden Delicious apple tree
5	Sovet farm, Merke	42º79'63.9"	73º20'80.2"	20.0	3.0	Starkrimson apple tree
6	Balabek farm, Merke	42º50'74.4"	73º08'54.3"	80.0	4.8	Idared apple tree
7	Baizhanova farm, Bayzak	42º50'74.8"	73º08'54.1"	79.0	4.7	Golden Delicious apple tree
8	Kargylash farm, Bayzak	42º50'74.8"	73º08'54.1"	81.0	4.9	Golden Delicious apple tree
9	Zhanat, Bayzak	42º84'58"	73º14'23.6"	80.0	4.9	Golden Delicious apple tree
10	Aisha-Bibi village	42°50'58.58"	71°14'36.47"	36.0	4.8	Quince
				34.0	4.7	Cherry plum
				77.0	4.6	Talgarskaya Krasavitsa pear tree
11	Pan farm, Korday	43º22'01.5"	74º45'84.9"	34.5	4.3	Golden Delicious apple tree
12	Kungey farm, Korday	43º11'58.4"	74º70'69.9"	21.0	0.8	Golden Delicious apple tree
13	Maksat-Tere farm	42º50'82.0"	70º30'70.3"	31.0	1.5	Golden Delicious apple tree

Note: P is the prevalence of the disease; R is the development of the disease.

Based on the analysis of samples for hypersensitivity and pathogenicity reactions from 339 strains, only 12 strains were identified as *E. amylovora* (nine strains on apple, three strains on pear) (Table 5).

To establish the species of the bacteria selected during the studies and confirm the already determined ones by the method of hypersensitivity and pathogenicity of 12 isolates of *E. amylovora*, the FLASH-PCR method was used. According to the study results, 77 isolates showed a positive result for *E. amylovora*, 17 isolates were negative, and seven isolates showed an unreliable result. During our study, we first discovered the manifestation of the disease on drupaceous cultures (plum and quince) in the Zhambyl region. Besides, in the Turkestan region, foci of the disease were detected on apricots and peaches.

Table 4. Results of phytosanitary monitoring of garden cenoses for fire blight pathogen infection in the Turkestan region of Kazakhstan, 2023.

No.	Name of the organization or farm	Latitude (N)	Longitude (E)	Disease index, %		Fruit culture, variety
				P	R	
1	Amangeldi LLP	41º60'19.4"	69º36'54.7"	2.4	0.3	Golden Delicious apple tree
2	Yntymak Ben Arys farm	41º52'36.3"	69º37'91.7"	3.6	0.7	Golden Delicious apple tree
3	Zhana kush farm	42º16'04.9"	69º53'82.2"	2.58	0.05	Golden Delicious apple tree
4	Alan and Company LLP	42º23'86.7"	69º23'84.9"	15.0	0.4	Golden Delicious apple tree
				39.0	4.0	Peachtree
				43.0	5.0	Talgarskaya Krasavitsa pear tree
5	Amangeldy farm	41º36'43.2"	69º22'84.4"	21.0	2.0	Golden Delicious apple tree
6	Birlik SEC	41º37'20.5"	69º21'21.0"	28.0	2.3	Golden Delicious apple tree
7	Saryagash Zher Syiy LLP	41º31'02.9"	69º23'59.0"	32.0	2.6	Leto plum tree
				14.0	0.5	Golden Delicious apple tree
8	Maksat Aray LLP	41º29'92.8"	69º20'32.2"	20.0	4.0	Golden Delicious apple tree
9	Alkhory farm	41º29'55.1"	69º17'78.9"	23.0	7.0	Golden Delicious apple tree
10	Kablanbek farm	41º29'13.1"	69º15'34.0"	27.0	5.0	Golden Delicious apple tree
11	An abandoned garden at the exit from Shymkent	42°16'42.06"	69°33'22.55"	58.0	14.8	Wild apricot
12	Ak Niet Agro Gardens LLP	41º62'03.8"	69º93'59.9"	44.0	3.8	Pink Lady apple tree
				15.0	0.8	Konfetka apple tree
				33.0	0.8	Idared apple tree
13	Keremet-Sapa LLP	42º23'73.3"	69º24'45.5"	24.0	3.0	Golden Delicious apple tree
14	Private plot at the Utepov village	41º46'86.1"	69º23'39.0"	25.0	6.0	Golden Delicious apple tree

Note: P is the prevalence of the disease; R is the degree of development of the disease.

Figure 4. Various organs of the Aport apple tree affected by fire blight in the Almaty region (left untreated as a control variant).

Figure 5. Hypersensitivity test on the leaves of the *P. domesticum* plant (a: control with no lesion; b: chlorosis).

a b

Figure 6. Pathogenicity test on unripe pears (a: control with no lesion; b: the presence of necrosis and exudate).

Table 5. Results of identification of the fire blight pathogen on fruit crops from plants of the *Rosaceae* family by the method of hypersensitivity and pathogenicity.

Breed	Samples analyzed	Obtained isolates	Identified as *E. amylovora*	Concomitant microflora
Apple tree	248	165	9	156
Peachtree	2	10	-	10
Quince	2	15	-	15
Cherry plum	1	10	-	10
Rose	1	9	-	9
Pear tree	10	81	3	78
Plum tree	7	32	-	32
Wild apricot	2	12	-	12
Parent plant of the ARM-18 clone rootstock	1	5	-	5
Total	274	339	12	327

3.2. Agar diffusion test

The Agar diffusion test showed that out of 156 bacterial isolates, antagonistic activity against *E. amylovora* 1E IMIV (to a greater or lesser extent) was detected only in 21 isolates, which are shown in Table 6.

Here, isolates with low activity (with pathogen growth inhibition zones less than 10 mm), medium activity (growth inhibition zones of 15-25 mm), or high activity (growth inhibition zones of 30 mm and more) were noted. The antagonistic activity of the isolates was noted on day 2, the inhibition zones of the pathogen 1E IMIV not only persisted but also increased over time. The maximum growth inhibition zones were recorded on day 7. As can be seen from Table 5, most of the isolates had an average inhibitory activity.

The greatest antagonistic activity was observed in two isolates 16.2 and 19.2, where, on day 7, the pathogen inhibition zones were 52.2 ± 2.58 mm and 45.6 ± 0.55 mm, respectively (Table 6). Isolates 16.2 and 19.2 were selected for molecular genetic identification.

3.3. Molecular genetic characteristics of microbial antagonists

As a result, the analyses carried out based on the obtained sequence of nucleotides of the 16SpRNA gene site established that all selected strains belonged to the *Pseudomonas* genus (Table 7, Figure 7).

As can be seen in Figure 7, isolates 16.2 and 19.2 are located in the same branch with *P. fluorescens*, *P. atacamensis*, *P. koreensis*, and *P. moraviensis*.

The results of genetic identification can be used as a molecular biological characteristic of strains.

4. Conclusions

Thus, in the course of our monitoring studies, foci of fire blight in fruit crops were identified in three regions of Kazakhstan. Isolates related to the fire blight pathogen *E. amylovora* were isolated and characterized. Microorganisms with antagonistic activity against *E. amylovora* were isolated from plant samples collected from the phyllosphere of the apple tree. Isolates 16.2 and 19.2 with high antagonistic activity were identified, in which the pathogen growth inhibition zones were 52.2 ± 2.58 and 45.6 ± 0.55 mm, respectively. According to the results of genetic identification, they belong to the *Pseudomonas* genus. In the future, the selected microbial antagonists can be used to create a new biological product in the fire blight control for fruit crops.

Table 6. Isolates of bacteria with antagonistic activity against *E. amylovora* obtained from apple tree plant samples.

No.	Isolate	The diameter of the growth inhibition zones of *E. amylovora* 1E IMIV, mm		
		2 days	5 days	7 days
	Turkestan region			
1	19.2	20.0 ± 2.0	35.8 ± 2.84	45.6 ± 0.55
2	9.5	3.1 ± 0.36	6.0 ± 1.5	9 ± 0.8
3	4.5	10.3 ± 1.52	13.5 ± 2.3	45.4 ± 0.51
4	3.8	17.0 ± 3.21	25.0 ± 1.5	25.0 ± 2.1
5	8.5	13.6 ± 1.51	27.0 ± 2.7	25.9 ± 5.3
6	1.6	9.3 ± 0.58	10.2 ± 0.73	14.8 ± 0.23
7	10.8	7.2 ± 0.75	11.5 ± 0.55	22.4 ± 0.89
	Zhambyl region			
8	1.9	14.7 ± 0.36	32.2 ± 2.75	25.3 ± 0.61
9	16.2	15.8 ± 0.82	27.9 ± 2.0	52.2 ± 2.58
10	5.6	7.5 ± 0.5	9.5 ± 1.4	10.7 ± 1.48
11	3.5	20.5 ± 1.32	25.0 ± 1.9	24.5 ± 1.8
12	4.5	9.4 ± 0.56	18.0 ± 1.5	25.2 ± 4.13
13	2.5	4.8 ± 0.66	7.7 ± 1.55	11.7 ± 1.75
14	1.5	3.9 ± 0.45	7.0 ± 1.1	24.5 ± 4.8
15	1.8	4.5 ± 0.32	9.0 ± 1.5	12.9 ± 2.75
	Almaty region			
16	10.6	5.8 ± 0.72	7.4 ± 0.55	9.0 ± 0.55
17	11.5	7.7 ± 1.25	10 ± 0.8	10.5 ± 0.55
18	9.6	3.4 ± 0.72	4.2 ± 1.3	10.0 ± 4.35
19	3.6	4.0 ± 0.5	4.0 ± 1.5	5.86 ± 0.78
20	2.6	6.3 ± 0.76	7.1 ± 1.4	7.63 ± 0.55
21	10.5	2.6 ± 0.55	4.8 ± 0.37	7.5 ± 0.5
22	Control: liquid SPA environment without bacteria	0	0	0
23	Control: liquid MRS medium without bacteria	0	0	0
24	Control: liquid FPA medium without bacteria	0	0	0

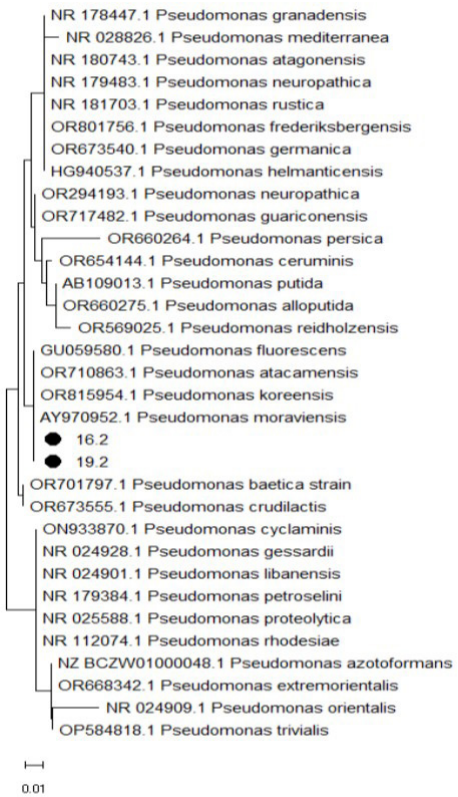

Figure 7. A phylogenetic tree based on analysis of the 16S rRNA gene fragment of samples 16.2 and 19.2.

Table 7. Results of identification by analysis of the nucleotide sequence of the 16S rRNA gene.

Name of the isolate	Sequence of the 16S rRNA fragment and ITS gene	Identification of nucleotide sequences in the international database (NCBI, 2023) BLAST algorithm		
		GenBank reference number (accession number)	Name of the strain	% of match
16. 2	ACCTCAGTGTCAGTATCAGTCCAGGTGGT CGCCTTCGCCACTGGTGTTCCTTCCT ATATCTACGCATTTCACCGCTACACAGGA AATTCCACCACCCTCTACCATACTC TAGCTTGCCAGTTTTGGATGCAGTTCCCA GGTTGAGCCCGGGGATTTCACATC CAACTTAACAAACCACCTACGCGCGCTTTA CGCCCAGTAATTCCGATTAACGCTT GCACCCTCTGTATTACCGCGGCTGCTGGCA CAGAGTTAGCCGGTGCTTATTCTGTC GGTAACGTCAAAACAGCAAAGTATTAATTTA CTGCCCTTCCTCCCAACTTAAAGTGC TTTACAATCCGAAGACCTTCTTCACACAC GCGGCATGGCTGGATCAGGCTTTCGCCC ATTGTCCAATATTCCCCACTGCTGCCTCCC GTAGGAGTCTGGACCGTGTCTCAGTTCC AGTGTGACTGATCATCCTCTCAGACCAGTT ACGGATCGTCGCCTTGGTGAGCCATTAC CTCACCAACTAGCTAATCCGACCTAGGCT CATCTGATAGCGCAAGGCCCGAAGGTCC CCTGCTTTCTCCCGTAGGACGTATGCGGT ATTAGCGTTCCTTTCGAAACGTTGTCCCC CACTACCAGGCAGATTCCTAGGCATTACT CACCCGTCCGCCGCTGAATCCAGGAGCAAGCTCC	MN826557.1	*Pseudomonas baetica* strain cqsm_h4 16S ribosomal RNA gene	100%
		MF079283.1	*Pseudomonas koreensis* strain NES-CAP-3 16S ribosomal RNA gene	100%
		KU990893.1	*Pseudomonas putida* strain I3 16S ribosomal RNA gene	100%
		KT350501.1	*Pseudomonas fluorescens* strain MFAF76a 16S ribosomal RNA gene	100%
		ON202871.1	*Pseudomonas helmanticensis* strain BZD_ww1 16S ribosomal RNA gene	100%
19.2	CGCACCTCAGTGTCAGTATCAGTCCAGGT GGTCGCCTTCGCCACTGGTGTTCCTTCCTATA TCTACGCATTTCACCGCTACACAGGAAATT CCACCACCCTCTACCATACTCTAGCTTGCCAGTT TTGGATGCAGTTCCCAGGTTGAGCCCGGGGAT TTCACATCCAACTTAACAAACCACCTACGCGCG CTTTACGCCCAGTAATTCCGATTAACGCTTGCA CCCTCTGTATTACCGCGGCTGCTGGCACAGAG TTAGCCGGTGCTTATTCTGTCGGTAACGTCAAAA TTGCAGAGTATTAATCTACAACCCTTCCTCCC AACTTAAAGTGCTTTACAATCCGAAGACCTTCTT CACACACGCGGCATGGCTGGATCAGGCTTTCGC CCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAG GAGTCTGGACCGTGTCTCAGTTCCAGTGTGAC TGATCATCCTCTCAGACCAGTTACGGATCGTCGCCT TGGTGAGCCATTACCTCACCAACTAGCTAATC CGACCTAGGCTCATCTGATAGCGCAAGGCCCGA AGGTCCCCTGCTTTCTCCCGTAGGACGTATGCG GTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACT ACCAGGCAGATTCCTAGGCATTACTCACCCG TCCGCCGCTGAATCCAGGAGCAAGCTCCCTTC ATCCGCTCGACTTGCATGTGTTAGGC	CP107544.1	*Pseudomonas moraviensis* strain EFBE32 chromosome	100%
		CP015852.1	*Pseudomonas koreensis* strain CRS05-R5 chromosome	100%
		CP077081.1	*Pseudomonas atacamensis* strain SWRI76 chromosome	100%
		JN679853.1	*Pseudomonas fluorescens* strain 1582 16S ribosomal RNA gene	100%

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