# CHARACTERIZATION OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* FROM DISEASED STONE FRUITS IN KYRGYZSTAN AND TESTING OF BIOLOGICAL AGENTS AGAINST PATHOGEN

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**A R T I C L E   I N F O**

**A B S T R A C T**

The plant diseases caused by the *Pseudomonas syringae* complex *b*acteria are economically important and occur worldwide on various plants, and it is as a pathogen that has not been the object of studies and little is known about its epidemiology in Kyrgyzstan. The conventional phenotypic (LOPAT, API tests) and PCR-assisted isolation were used for the identification of *Pseudomonas syringae* pv. *syringae* isolates from the affected organs of local stone fruits, such as peach (*Prunus persica*), cherry (*Prunus subgen*), apricot (*Prunus armeniaca),* and plum (*Prunus salicina*) samples taken from the Chy, Issuk-Kul, and Batken regions of the country. 16S rRNA gene amplification was performed with primers 27F (5'-AGA GTT TGA TCC TGG CTC AG -3') and 907R (5 '–CCG TCA ATT CCT TTG AGT TT-3') for the identification of obtained *P.syringae* pv. *syringae* isolates. From 40 primary isolates of Gram-negative rod-shaped bacteria, 12 were identified as *Pseudomonas syringae pv. syringae, while the remaining* isolates were identified as bacteria from *Stenotrophomonas, Xanthomonas, Erwinia* genera. The antagonist bio control agent—*Streptomyces b*acteria strains were screened and selected against the bacterial canker pathogen in *in vitro* experiments and on apricot seedlings *in vivo* conditions. Obtained results could encourage to develop a local bio-product based on this bioagent for spraying stone fruits with the initial manifestation of disease symptoms and to conduct preventive treatments in the fall and spring to increase the plant's resistance to pathogens.

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## INTRODUCTION

The plant diseases caused by the *Pseudomonas syringae* complex bacteria are economically important and occur worldwide on various plants. Among plant pathogenic bacteria, P*seudomonas*, pv. *syringae* can cause diseases in more than 180 plant species including annual and perennial plants, fruit trees, ornamentals, and vegetables (Little *et al.*, 1998; Agrios, 2005; Lamichhane *et al.*, 2014; Lamichhane *et al.*, 2015). The phytopatogenic pseudomonads cause plant diseases with different symptoms, including cankers, diebacks, blossom, twig, leaf or kernel blights, leaf spots (*P. syringae* different pathovars), soft and brown rots (*P. viridiflava, P. marginalis* different pathovars), tumors or galls (*P. savastanoi* pathovars), mushroom blights (*P. tolasii, P. agarici* pathovars), and tomato *(P. syringae* pv*. tomato*) (Koike *et al.*, 2017; Braun-Kiewnick and Sands, 2001; Choi *et al.*, 2016; Ahmed *et al.*, 2018).

Meanwhile, other authors noted that *P. syringae* strains form an overlapping continuum of host range potential with equal representation of narrow, moderate, and broad host ranges (Morris *et al.*, 2019). Several examples

have been observed for the overlapping of this pathogen bacteria, in that sugar beet and cucurbits are considered as common hosts for *P. syringae* strains (Morris *et al.*, 2000; Sedighian *et al.*, 2014). *P. syringae* pv. *garcae* is not a coffee pathogen but can affect coffee seedling (Destéfano *et al.*, 2010).  *P. syringae* pv. *lachrymans* is a pathogen of the ornamental tree White Bird of Paradise (*Strelitzia augusta*), which has shown a virulence on zucchini (Polizzi *et al.*, 2005).

*P. syringae* bacteria as the ubiquitous pathogen of numerous plant species has a special name—*P. syringae sensu lato*—proposed by (Morris *et al.*, 2013). This group consists of a phylogenetic line containing related species *P. syringae* (Mulet *et al.*, 2010). The multilocus sequence analysis (MLSA) (Berge *et al.*, 2014) allowed  to classify the metapopulation of *P. syringae* strains involved in various agricultural plant diseases (Lamichhane *et al.*, 2014; Lamichhane *et al.*, 2015). *P. syringae* is composed of plant pathogens divided into 60 pathovars (Young, 210) belonging to nine genomospecies, as determined by DNA: DNA hybridization (Gardan *et al.*, 1999).

The diagnostics of bacterial canker are commonly based on isolation and the phenotypic characterization of the causal agent, including pathogenicity (Bultreys and Gheysen, 1999; Vicente *et al.*, 2004). The phenotypic tests LOPAT (Lelliott *et al.*, 1966; Lelliott and Stead, 1987), GATTa ,and L-lactate utilizations (Latorre and Jones, 1979) enable the determination of morphological, physiological, and biochemical characteristics of the bacteria. On King's B medium, the majority of these bacteria produce a fluorescent pigment visible under UV light (King *et al.*, 1954).These tests are used for the identification of species and their discrimination into pathovars and races.

To protect plants from diseases caused by *P. syringae* pathogen, such bio-control bacteria like *P. aeruginosa* strain D4, *Bacillus stratosphericus* strain FW3, *Paenibacillus polymyxa* AC -1 were screened *in vitro* and *in vivo* trials. *Pseudomonas aeruginosa* strain D4 and *Bacillus stratosphericus* strain FW3 isolated from mine tailings in South Korea have shown improved antagonistic activity against *P. syringae* DC3000. The bio-control efficiency of the potential strains on the *S. lycopersicum* plant against *P. syringae* pathogen was resulting in growth promotion  and *in vivo* antagonistic activity (Durairaj *et al.*, 2018).

The biocontrol ability of a wild-type *B. subtilis* strain 6051 against the bacterial pathogen *P. syringae* pv.

*tomato* DC3000 infecting *Arabidopsis* roots was evaluated *in vitro* and in soil. The minimum inhibitory concentrations of *B. subtilis*  were relatively high (25mg mL $^{-1}$) to be sufficient to kill *P. syringa*e (Bais *et al.*, 2003). *Paenibacillus polymyxa* AC-1 rhizobacterium has been  examined for effects on bacterial phytopathogen *P. syringae* and  this biocontrol agent  has inhibited the growth of both *P. syringae*  pv. *tomato and P. syringae* pv. *tabaci*  in  a concentration-dependent  manner *in vitro* assays (Hong *et al.*, 2016). *P. fluorescens* biocontrol bacteria isolated from flowers of *Actinidia deliciosa* and *A. chinensis* were tested against *P. syringae* pv. *actinidiae* (Psa), the causal agent of kiwifruit bacterial canker  and were able to inhibit Psa growth *in vitro* and to reduce disease incidence in planta (Donati *et al.*, 2018).

Bacterial canker is the most harmful disease of the cultivated varieties of apricots, plums, cherries, peaches, and ornamental *Prunus* species in Kyrgyzstan. It causes sunken patches of dead bark and small holes in leaves, called "shothole". *P. syringae* pv. *syringae* is a pathogen that has not been the object of studies and little is known about its epidemiology in Kyrgyzstan. Additional problem is a limited number of measures are available to control bacterial plant diseases. A better understanding of the disease epidemiology would help limit its incidence by providing more information on the correct timing for the application of protective sprays.

This study aimed to isolate, identify, and screen the *P. syringae* group bacteria using the widely used and still highly sensitive culture approach, and by the PCR analysis of 16S rRNA gene for the identification of obtained natural isolates of *P. syringae* and select and develop biocontrol agents for the protection of stone fruit orchards from bacterial canker.

## METHODS AND MATERIAL
### Plant Sample Collection
In spring 2015–2017, early summer, and autumn expeditions were organized to different stone fruit growing regions of the country. The samples were taken from different varieties of apricot (*Prunus armeniaca*), peach (*Prunus persica)*, plum (*Prunus salicina),* and cherry (*Prunus subgen*) with disease symptoms in Chy, Batken, and Issyk-Kul valleys to isolate the bacterial canker pathogen isolates. An additional one hundred samples (leaves, stems, spurs, and bark with gummy ooze) from 14 orchards were collected (Table 1).

Table 1. Samples taken from diseased plants.

| Time of sampling | Sampling place, GPS coordinates | Plant species | Plant organs |
|---|---|---|---|
| The end of April and May 2015, and October 2015 and 2017 | Botanical Garden named after Gareev, Bishkek city 42°52'12'' N 74°35'24'' E | Different varieties of apricots, plums, and peaches | The affected shoots, leaves, barks ,and fruits |
| May 2015, and October 2015, 2016, and 2017 | Bishkek city 42°52'12'' N 74°35'24'' E | Different varieties of apricots and plums, cherries | The affected shoots and leaves |
| The end of April and May 2015, and October 2015, 2016, and 2017 | Chui region, Vorontsovka, Kokzhar villages 42°49'11'' N 75°16'56.4'' E | Different varieties of apricots, peaches, and plums, cherries | The affected shoots, leaves, barks, and fruits |
| October 2015, 2016, and 2017 | Chui region, Ak-Beshim wild apricot orchard 42°53'4.3'' N 74°40'41.5'' E | Wild varieties of apricots , cherries | The affected shoots and leaves |
| October 2016, and May 2017 | Issyk-Kul region, Fruit farming of Balykchy, Bokonbaevo villages 42°7'3.7'' N 76°59'35.9'' E 42°27'44.9'' N 76°11'23.9'' E | Different varieties of apricots | The affected shoots, leaves, and barks |
| October 2016 and May 2017 | Issyk-Kul region, fruit farming of Tosor, Zharkynbaevo, Khajisai, and Zhargylchak villages 42°9'56.5'' N 77°26'39.8'' E 42°8'17.5'' N 77°10'38.2'' E | Different varieties of apricots | The affected shoots , leaves, barks, and fruits |
| October 2016 | Batken region, Apricot farming villages 39°46'6.6'' N 69°27'50.3'' E | Different varieties of apricots | The affected shoots leaves, and fruits |

**Extraction of bacteria from primary materials**

Samples were washed and cut in pieces in a sterile bag, then placed in a suitable container such as a disposable 150 ml plastic cup with lid or a 200 ml Erlenmeyer flask. Furthermore, 30 ml phosphate-buffered saline was added. Subsequently, the container was placed on a rotary shaker and incubated at 200 rev/min for 1h. For samples with symptoms, an appropriate amount of macerate was selected for the polymerase chain reaction (PCR) analysis. As regards asymptomatic samples, extracted suspension was concentrated by centrifugation, and 50 ml of macerate was carefully poured either directly into the centrifuge tube—leaving the pulp in a container—or pre-filtered through filter paper and then centrifuged for 10 minutes, with an acceleration of 8000 g. The sample was also frozen at -18 ℃.

The supernatant was discarded without damage; the pellet was resuspended in 1 ml of phosphate buffer and transferred to a sterile microtube. The extract was then used immediately for the selection tests: biochemical and PCR analyses (Bultreys and Gheysen, 1999; Braun-Kiewnick and Sands, 2001; Stefani and Loreti, 2014).

**Mediums for isolation and characterization of _Pseudomonas syringae_ bacteria**

Differential and semi-selective mediums were used for the characterization of colonies based on the color, texture, and other cultural features.

_Pseudomonas_ Isolation Agar (Millipore, Sigma-Aldrich) consists of: agar, 11.6 g/L; magnesium chloride $6H_2O$, 1.4 g/L; peptic digest of animal tissue, 16.0 g/L; casein enzymic hyrolysate, 10.0 g/L. This medium was used for primary isolation and identification of _Pseudomonas_ bacteria from plant samples.

King Agar B medium (Millipore, Sigma-Aldrich) consists of: peptone 5 g; glycerol 10 ml; $K_2HPO_4$, 1.5 g; $MgSO_4.7H_2O$-1.5 g; agar 20 g; distilled water 1 L; pH 7.0–7.2; sterilized by autoclaving at 120 ℃ for 20 min. This medium was used for density growth, fluorescing and producing yellow-green pigment colonies.

Levan medium (Millipore**,** Sigma-Aldrich) consists of: yeast extract-5 g; bactopeptone-5 g; NaCl, 5 g; sucrose 50 g; agar 20 g; distilled water 1 L; pH 7.0–7.2; sterilized by autoclaving at 120 ℃ for 20 min. This medium was used for levan or a polyfructan production by _P. syringae._

Glucose Peptone Agar (Millipore, Sigma-Aldrich) consists of: peptic digest of animal tissue 20.0 g/L, dextrose 10.0 g/L; sodium chloride 5.0 g/L; agar 15.0 g/L, final pH (at 25 °C) 7.2±0.2. This highly nutritious medium was used to support the growth of fastidious microorganisms, like *P. syringae and* for general cultivation.

Nutrient agar (Millipore, Sigma-Aldrich). Ingredients (g/L): Peptic digest of animal tissue 5.0 g Beef extract 3.0 g, Agar 15.0 g. This composition was used for the cultivation of isolated bacteria.

### Isolation of the pure culture of *Pseudomonas syringae*

In order to isolate the *P. syringae* isolates from prepared primary materials as well as identify and store them in vitro, the selective mediums such as *Pseudomonas* Isolation Agar; King's B medium (King *et al.*, 1954), Levan, Glucose peptone agar (GPA) and nutrient agar (NA) with sucrose were used. A 30-50 μl dose of plant extract suspension from the diseased parts was added to the Petri dish and sequentially distributed over the surface of the King's B medium on three plates with a spatula. The stroke method was also used for plating the plant extract suspension on Pseudomonas Isolation Agar. For this purpose, four decimal dilutions of plant extract were prepared in the extraction buffer followed by 50-100 μl of undiluted extract, before each dilution was plated on its medium with the stroke method. In order to control the quality of the medium, the reference strains of bacterial canker pathogen were plated. After 28h of incubation at 28 °C, Pseudomonas such as colonies were purified twice on King's B medium before being characterized. All the strains were maintained in King's B medium at 4 °C for collection and other biochemical tests.

### The classical biochemical tests

Biochemical tests were set for the study of phenotypic properties of the *P. syringae* isolates based on LOPAT (levan production, oxidase test, potato rot, arginine dihydrolase, and tobacco hypersensitive reaction) and GATTa's (gelatin liquefaction, aesculin hydrolysis, tyrosinase activity, and Na-tartrate utilization) group tests (Lelliott *et al.*, 1966; Lelliott and Stead, 1987).

### The hypersensitivity of plant tissue to *Ps. syringae*

*In vitro* bioassay tests to identify the hypersensitivity of non-specific plant organisms to isolated bacterial canker

pathogens were performed. Tobacco plants (*Nicotiana tabacum var Hicks)* were grown in a greenhouse for eight to 12 weeks after transplanting. Fully expanded upper leaves were inoculated by injecting bacterial suspensions of $1 \times 10^8$ viable bacteria/ml into the intercellular spaces. The cells were washed using 3.4 ml of saline and 15–20 μl was injected into the pulp of leaves with a sterile syringe. As a control, the same volume of sterile physiological saline was injected (Johansson *et al.*, 2015).

### Artificial infection of pear, cherry, and plum fruits to determine the *Ps. syringae* virulence

The most common and simple White method was used for the identification of the degree or strength of the virulence of the locally obtained isolates. In these tests, the immature and mature pear, cherry, and plum fruits were inoculated with *P. syringae* liquid culture ($10^9$ of cells/ml), and the fruits were kept in a humid chamber. In control, the fruits were inoculated with sterile water. The results were estimated by a 5-point scale.

### Temperature effects on the in vitro growth of *Ps. syringae* isolates

The *Ps. syringae* isolates were cultivated in peptone meat broth on the shaker (200 rev/min) at the different temperatures of 15, 20, 28, and 40 °C for 48 hours of incubation. The optical density of bacterial suspension was measured at 525 nm (Janyway, UK) and a standard curve was created. The number of bacteria was subsequently counted.

### Assessment of the antibiotic impact on the *Ps. syringae* isolates

During this experiment, YGA and YPA mediums were used. The molten medium was mixed with the bacterial culture and poured into Petri dishes. After cooling, the wells of 10 mm in diameter were made in the medium. These wells were filled with antibiotic solutions (erythromycin, ofloxacin, penicillin, and tetracycline) of 50 mg/ml, 100 mg/ml, 150 mg/ml, and 200 mg/ml doses respectively, and incubated at 28 °C for 48 h. The appearance of lysis zones around the wells proved the sensitivity of pathogen cultures to various antibiotics.

### *In vitro* determination of antibiotic activity of antagonistic microorganisms against the pathogen

The agar blocks method was used. Biocontrol

antagonistic microorganisms (*Streptomyces sp.*) were plated onto the surface of the agar medium in a Petri dish, having formed during the growth of a continuous lawn. Cultures were incubated at a suitable temperature for four to five days. Then a sterile cork drill (6-8 mm in diameter) was used to cut from the layer agar blocks and transferred to the surface of the agar medium, inoculating only the test organism (*P. syringae*). The appearance of lysis zones around the wells has proved the sensitivity of pathogen cultures to *Streptomyces* biocontrol strains. Total of six *Streptomyces* strains were tested against *P. syringae* isolates; the tested experiments were performed in triplicate.

**Screening the antibiotic activity of antagonistic microorganisms against the bacterial canker pathogen on apricot seedlings**
The potential of candidate antagonists to suppress the cell production of *P. syringae* on infected leaves was tested on susceptible local apricot seedlings. Seedlings were sprayed with suspensions of *P. syringae* ($1 \times 10^6$ ml$^{-1}$) until runoff and seedlings were incubated for five days at 85% RH, 15 °C and with 16 h. of light per day. Thereafter, *P. syringae* inoculated seedlings were sprayed with antagonist suspensions (containing $1 \times 10^6$ spores or cells ml$^{-1}$) or water (containing 0.01% Tween 80) as controls. Two seedlings of both apricots were used for each replicate of each treatment. Contact between leaves of neighbouring plants was avoided. Seedlings were grown for 9 to 12 days at 15 °C, with 16 h of light per day at 138 $\mu$E s$^{-1}$ m$^{-2}$. From both seedlings of each replicate, the youngest five true leaves were carefully removed, put into Duran bottles (100 ml) containing 35 ml of tap water with 0.01% Tween 80. Four, 10, 15 and 20 days after treatment with antagonist cultures, five leaves of the apricot seedling were removed for microscopy and planting on the relevant mediums.

**Molecular Identification**
**Sample preparation for polymerase chain reaction**
King's B medium-positive, fluorescent culture at a concentration of $10^6$ cells/ml in sterile distilled water suspension was prepared and used immediately or stored at -18 °C until the PCR product was observed.
**Polymerase chain reaction**
Total DNA was isolated with a cell using DNeasy Blood Tissue Kit according to standard protocols of QIAGEN,

Germany**.** Amplification was performed with a Multigene Thermal Cycler (TC9600-G/TC, Labnet International), using a 25 $\mu$l mixture containing 15 µl of PCR Master Mix (*Taq* DNA polymerase, MgCl$_2$, deoxy-ribonucleotide triphosphate and reaction buffer), 2 µl of each primer, 1 µl of template DNA, and 1 µl of H$_2$O. The amplification program was used as follows: 95 °C for 5 min, followed by 30 cycles of 1 minute denaturation at 95 °C, 1 minute annealing at 55 °C, 1 minute extension at 72 °C, and a final extension step of 5 minutes at 72 °C. The PCR products were electrophoresed in a 2.0 % agarose gel and visualized using the BioDoc-It$^{TM}$ Imaging Systems (Ultra-Violet Products Ltd.) after ethidium bromide staining. In order to control contamination, we used a negative control reaction and sterile water was added as a matrix.

**Statistical analysis**
In order to determine the difference between the disease index in plants and the inoculation methods (direct versus indirect), the variables were analyzed through a two-way ANOVA analysis using the SPSS v. 23.0 software (SPSS Inc.). The difference between the symptom score and the two variables was also determined with the same analysis. The effects were significant at $P \leq 0.05$.

**RESULTS**
**The survey results**
For 2015–2016, the survey was carried out in the Kokzhar, Vorontsovka, and Ak-Beshim villages and Bishkek city of the Chy region. In spring and early summer, observations revealed the following symptoms o**n** stems and spurs: sunken, dead areas of bark that often accompanied by gummy ooze. If the infection had spread all-round, the branches died rapidly. On leaves of apricots and plums, small brown spots appeared which were often round and fell out later through leave holes (shothole). Bacterial canker can sometimes kill larger branches or whole trees (Figure1, A and B). As such, the infected fruit often develop depressed spots with dark centers and sometimes have underlying gum pockets. Ulcers and cracks on the bark reached up to one and a half meters. The degree of damage was estimated on a 5-point scale, and the lesions were about 2 points. While in the plantations of wild apricots, (Ak-Beshim village) the degree of damage did not exceed 1 point. In observed sites *P. syringae*, pv. *syringae* isolates were obtained from infected organs of pome trees like Peach (*Prunus persica*), Cherry (*Prunus subgen*), Plum (*Prunus salicina*).

The disease incident caused by *P. syringae* pv. *syringae* has reached to 80-90±0.093% (P≤0.05) in these analyzed trees. In addition, *Xanthomonas* sp. and *Stenotrophomonas* sp. were isolated from diseased part of trees, constantly accompanying the main pathogen. Such types of disease as bacterial leaf shot hole, bacterial fruit shot hole, bacterial canker, fruit necrosis and fruit canker were dominated (Table 2).

Table 2. Disease outbreaks on pome plants caused by the *P. syringae* pv. *syringae* complex reported in 2015-2017 in different regions of Kyrgyzstan.

| Region | year | Host trees | Disease | Pathogen | DI (%) | Concomitant bacterial species |
|---|---|---|---|---|---|---|
| Batken | 2016 | Apricot *(Prunus armeniaca)* | Bacterial leaf spot | *P. s.* pv. *syringae* | 80 | *Erwinia sp.* *Stenotrophomonas sp.* |
| Batken | 2016 | Apricot *(Prunus armeniaca)* | Stem necrosis | *P. s.* pv. *syringae* | 60 | *Stenotrophomonas sp.* *Erwinia sp.* |
| Batken | 2016 | Apricot *(Prunus armeniaca)* | Fruit necrosis | *P. s.* pv. *syringae* | 60 | *Stenotrophomonas sp.* *Pseudomonas sp.* |
| Chui region | 2015, 2016 | Peach (*Prunus persica*) | Bacterial leaf shot hole | *P. s.* pv. *syringae* | 80 | *Xanthomonas sp.* *Erwinia sp.* |
| Chui region | 2015, 2016 | Peach (*Prunus persica*) | Bacterial Canker | *P. s.* pv. *syringae* | 60 | *Pseudomonas sp.* *Xanthomonas sp.* |
| Chui region | 2015, 2016 | Cherry (*Prunus subgen*) | Bacterial leaf shot hole | *P. s.* pv. *syringae* | 80 | *Pseudomonas sp.* *Stenotrophomonas sp.* |
| Chui region, | 2015, 2016 | Cherry (*Prunus subgen*) | Bacterial Canker | *P. s.* pv. *syringae* | 90 | *Pseudomonas sp.* *Erwinia amylovora* |
| Bishkek city | 2015, 2016 | Plum (*Prunus salicina*) | Fruit necrosis | *P. s.* pv. *syringae* | 80 | *Xanthomonas sp.* *Erwinia sp.* |
| Bishkek city | 2015, 2016 | Plum (*Prunus salicina*) | Fruit Canker | *Xanthomonas sp.* | 80 | *Xanthomonas sp.* *Pseudomonas sp.* |
| Issyk-Kul region | 2016, 2017 | Apricot *(Prunus armeniaca)* | Bacterial Canker on the trunk, limbs | *P. s.* pv. *syringae* | 90 | *Pseudomonas sp.* *Erwinia sp.* |
| Issyk-Kul region | 2016, 2017 | Apricot *(Prunus armeniaca)* | Fruit Canker | *P. s.* pv. *syringae* | 90 | *Pseudomonas sp.* *Erwinia sp* |
| Bishkek city | 2015-2017 | Plum (*Prunus salicina*) | Bacterial leaf shot hole | *P. s.* pv. *syringae* | 90 | *Pseudomonas sp.* *Erwinia sp* |
| Bishkek city | 2015-2017 | Apricot *(Prunus armeniaca)* | Bacterial Canker on the trunk, limbs | *P. s.* pv. *syringae* | 90 | *Pseudomonas sp.* *Erwinia sp.* |

DI= Disease Incidence, *P. s.* pv. *syringae* = *Pseudomonas syringae* pv. *syringae*

During 2016–2017, in the Issyk-Kul region, the diseases were found in old apricot trees (*Prunus armeniaca*), but the greatest distribution was observed in Tosor village, where almost every tree was affected by bacterial canker, and even young seedlings were injured. When cutting the damaged stem, the outer layers of the wood were often stained a brown color in contrast to the normal white, but the inner wood (xylem) was not discolored except in the more severe cases (Figure 1C and D). The degree of damage was estimated: in orchards of Balykchi on a 3-point scale, Bokonbaevo on 1 point, and Tosor on 4 points, while Khadjisai and Zhargylchak on 3 points.

The disease incidence caused by *P. syringae* pv. *syringae* has reached up to 90% (P≤ 0.05) in the analyzed diseased part of apricot trees. The symptoms of bacterial canker on the trunk and limbs, fruit canker were strongly differed in unarmed observation and dominated among other symptoms. Along with the main causative agent, other bacteria species like *Pseudomonas* sp. *Erwinia* sp. were isolated into a pure culture; they can be considered as saprophytic or facultative pathogens

species that occupy a specific niche in the phyllosphere of trees (Table 2).

In the Batken region, the rapid development of this disease was observed during 2015–2016, and the degree of damage was estimated on a 3-point scale. Bacterial leaf spot, stem necrosis, fruit necrosis were the most spread diseases in these served sites. The disease incident caused by *P. syringae* pv. *syringae* has made about 60-80 ±0.092% (P≤0.05) in the analyzed diseased part of apricot trees. *Stenotrophomonas* sp. *Erwinia* sp. bacteria were isolated with *P. syringae* pv. *syringae* isolates frequently.
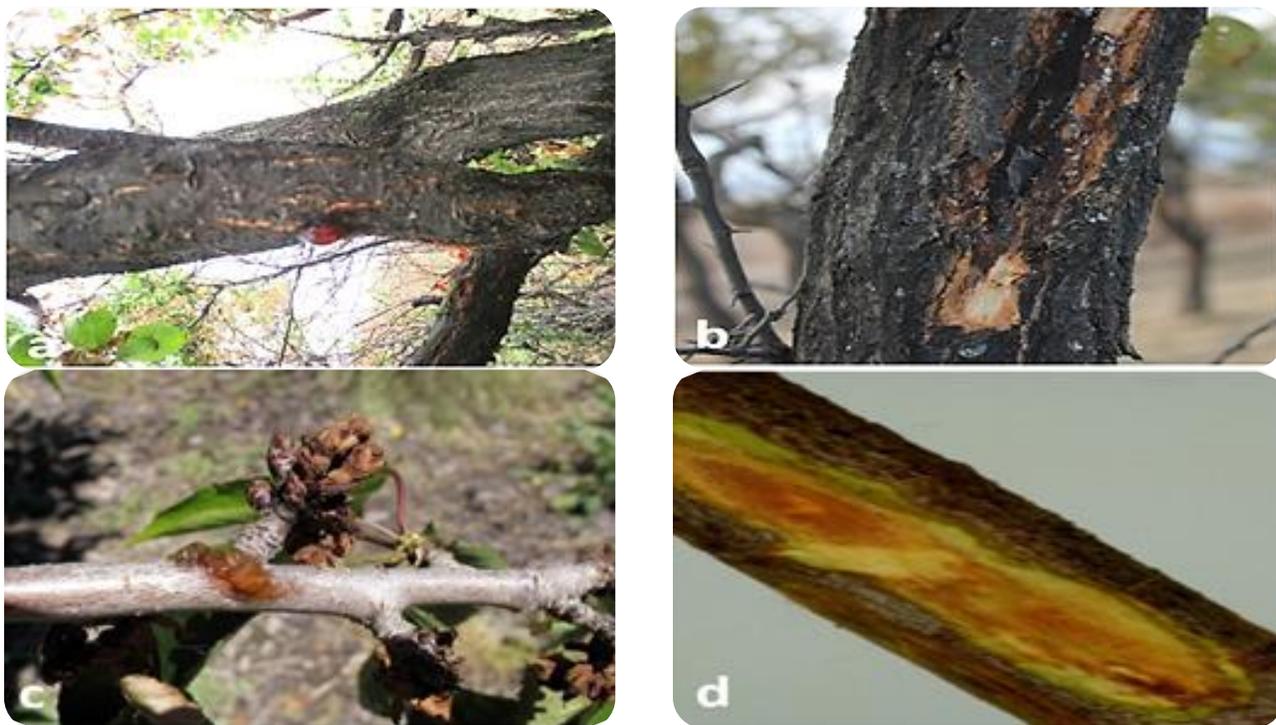


Figure 1: Symptoms of bacterial canker as shown in the diseased barks of old apricot trees (a, b) and of young apricot trees (c, d).

## The phenotypic characterization of *Pseudomonas syringae* pv. *syringae* isolates

A total of twelve *P. syringae* pv. *syringae* isolates have been detected to their biochemical and pathogenic properties and the prevalence of these bacteria in a phylloplane environment indicates their competence longevity (Table 3).

## The characterization of colonies formation and cultural properties on different media

All obtained isolates on King B medium formed typical for the species *P. syringae* pv. *syringae* colonies: roundish, whitish-gray, up to 5 mm in diameter, with a conical-raised center, a shaft-shaped slightly-wavy edge, semi-translucent, with a denser center and a smooth or slightly granular structure (O-form) (Figure 2 A and B). *P. syringae* J-5 strain on Levan medium plates: at room light and at UV light. As shown in Figure 2 (C and D),

bacterial cancer pathogen, such as the bacterial fire blight, has formed white colonies on the Levan medium. In order to differentiate between these pathogens, the UV light is required. Ps. syringae bacteria affected by this light have produced blue-green pigment, and while *Erwinia amylovora* in the presence of UV light remains unchanged. Figure 3 has shown the colonies *P. syringae* isolated from Plum (*Prunus salicina*) on different mediums.

The phytopathogenic *Pseudomonas* strains were identified according to Lelliott *et al.* (1966), who devised a determinative scheme that based on simple laboratory tests. The average growth time of the *P. syringae* isolates from apricot, plum, cherry and peach under laboratory conditions was 1 and 3 hours at 28 °C. All isolated strains have grown very intensely at 15 °C; especially the two strains of BT-5 and A2.2 that indicated to ability not lose their virulence at 15 °C.

Table 3: Characteristics of *Pseudomonas syringae* colonies growing on different mediums.

| Sr. | *P. syringae* strains | Source plant for isolation | GPA medium | Levan medium | King B medium |
|---|---|---|---|---|---|
| 1 | J-5 | Apricot *Prunus armeniaca* | Dry and dirty white color colonies | Yellow color colonies | Pale color, rough colonies |
| 2 | BO | Apricot *Prunus armeniaca* | Yellow colonies | Mucous, gold like colonies | Mucous yellow |
| 3 | SCH | Peach (*Prunus persica*), | Yellow colonies | Mucous yellow | Mucous yellow colonies |
| 4 | C2.3 | Plum (*Prunus salicina* | Rough yellow colonies | Yellow colonies | Dry and dirty white rough colonies |
| 5 | BT-5 | Cherry (*Prunus subgen*) | Dirty white color colonies | Mucous yellow colonies | Mucous yellow colonies |
| 6 | A2.2 | Apricot *Prunus armeniaca* | Small dot-like colonies | White small dot colonies | Dry and dirty white rough colonies |
| 7 | SCH-2 | Peach (*Prunus persica*) | Yellow colonies | Mucous yellow colonies | Mucous yellow colonies |
| 8 | K-4 | Cherry (*Prunus subgen*) | Yellow color colonies | Light yellow colonies | Mucous yellow colonies |
| 9 | AT-2 | Cherry (*Prunus subgen*) | Dairy-like, spherical colonies | Yellow color colonies | Dry and dirty white rough colonies |
| 10 | C2.5 | Plum (*Prunus salicina* | Rough yellow colonies | Yellow colonies | Dry and dirty white rough colonies |
| 11 | A1.4 | Apricot *Prunus armeniaca* | Dirty white colonies | Pale yellow colonies | Dry and dirty white rough colonies |
| 12 | C2.5 | Plum (*Prunus salicina* | Rough yellow colonies | Yellow colonies | Dry and dirty white rough colonies |
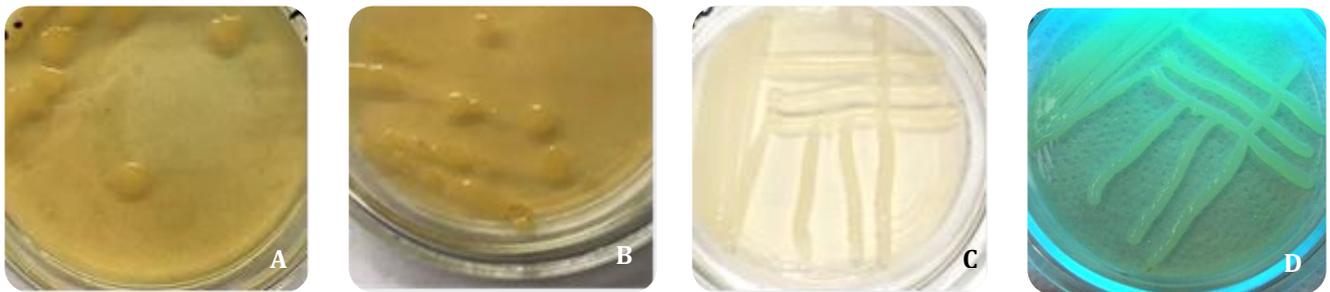


Figure 2. Colonies of bacterial canker pathogen on King B medium: A –colonies of *P. syringae* sp. isolates from Batken apricot; B – colonies of *P. syringae* sp.isolates from peach tree. C- colonies of *P. syringae* sp. isolates on the Levan medium, at room light; D- colonies of *P. syringae* sp. isolates on the Levan medium, at UV light.

**The biochemical characterization of *Pseudomonas syringae* isolates**

Phytopathogenic Pseudomonas are divided into pathotypes according to five biochemical tests. This test is abbreviated as the LOPAT test.

1. Levan formation (L); 2. Oxidase production (O); 3. Maceration of plant tissue (P) 4. Arginine dihydrolase production (A); 5. Hypersensitive reaction in tobacco (T)

There are fifty *P. syringae* pathotypes distinguished according to the above tests (L +, O-, P-, A-, T +), which cause diseases in 180 plant species (Lamichhane *et al.*, 2014; Lamichhane *et al.*, 2015).

Based on LOPAT (levan production, oxidase test, potato rot, arginine dihydrolase and tobacco hypersensitive reaction) and GATTa's (gelatin liquefaction, aesculin hydrolysis, tyrosinase activity and Na-tartrate utilization), group tests were identified with the phenotypic (biochemical and physiological)

characteristics. Almost all the studied pathogen strains were able to liquefy gelatin moderately; the liquefaction process started in five days and produced hydrogen sulphide (Table 4). However, all strains had simple nutritional requirements and may have utilized many

carbon sources such as glucose, succinate, glycerol, fumarate, and pyruvate as well as simple amino acids and small peptides. This broad diet may allow the colonization of diverse host plants as well as survival in water including rain, snowmelt, streams, and rivers.



Figure 3: The colonies of *Pseudomonas syringae* bacterium isolated from Plum (*Prunus salicina)*: on GPA medium ( a); on the Levan medium (b) ; on Ps. agar medium ( c).

Table 4: Biochemical tests for *Pseudomonas syringae* bacterium (n=3).

| S. # | Test name | *Pseudomonas syringae* isolates | | | | |
|---|---|---|---|---|---|---|
| | | BO | SCH | C.2.3 | BT-5 | A2.2. |
| 1 | Reduction of sugar compounds | + | - | - | - | - |
| 2 | Catalase test | + | + | + | + | + |
| 3 | Salinity resistance | + | + | + | + | + |
| 4 | Pigment production | - | - | + | - | + |
| 5 | $H_2S$ production | - | - | ? | ? | ? |
| 6 | Levan formation | + | + | + | + | + |
| 7 | Maceration of potatoes tuber | - | - | - | - | - |
| 8 | The growth on yeasts extract medium with $CaCO_3$ (YDC). | + | + | + | - | - |
| 9 | The growth on King B medium | + | + | + | + | + |
| 11 | Pectate degradation | + | + | + | - | - |
| 12 | Gelatin liquefaction | + | + | + | + | + |
| 13 | Hypersensitive reaction in tobacco (*Nicotiana tabaccum* L.) | + | + | + | + | + |
| | | *Ps.* pv. *syringae* | *Ps.* pv. *syringae* | *Ps.* pv. *morsprunorum* | *Ps.* pv. *syringae* | *Ps.* pv. *syringae* |

Most phytopathogenic bacteria can produce several pectolytic enzymes, each of which can be encoded by a family of genes and exist in several isoforms that differ in the optimum pH, temperature, and other properties. In our cases, the following strains were grown well on a pectin-containing medium: BO, K-4, SH-2 (Supplem. material, Figure 1, A, B, C and D). Almost all of obtained *P. syringae* isolates were grown well giving density colonies on hawthorn containing agar, some isolates were grown on currant containing media, nevertheless,

on this medium, isolates could increase the mass of their colonies, maintaining their viability for several days. These results prove the presence of lyase pectate in *P. syringae* isolates, which is the main enzyme that destroys pectin FI binding in plant tissues.

**The growth of *Pseudomonas syringae* isolates on media with different pH**
All the obtained isolates were able to grow in culture media with pH 6.0; 6.5; 7.0; 7.5, giving enough thick,

massive columns. As the data show (Figure 4 and Supplement. mater. Figure 4), all isolates within 48 hours giving an intensive 100% growth on solid medium and in liquid medium with all the above-mentioned pH values. That indicating in the above-

mentioned pH values, they can absorb nutrients and carry out normal metabolism. Such features of metabolism prove that these bacteria can infect fruit trees in all phases of development; young, developing, and ripe.
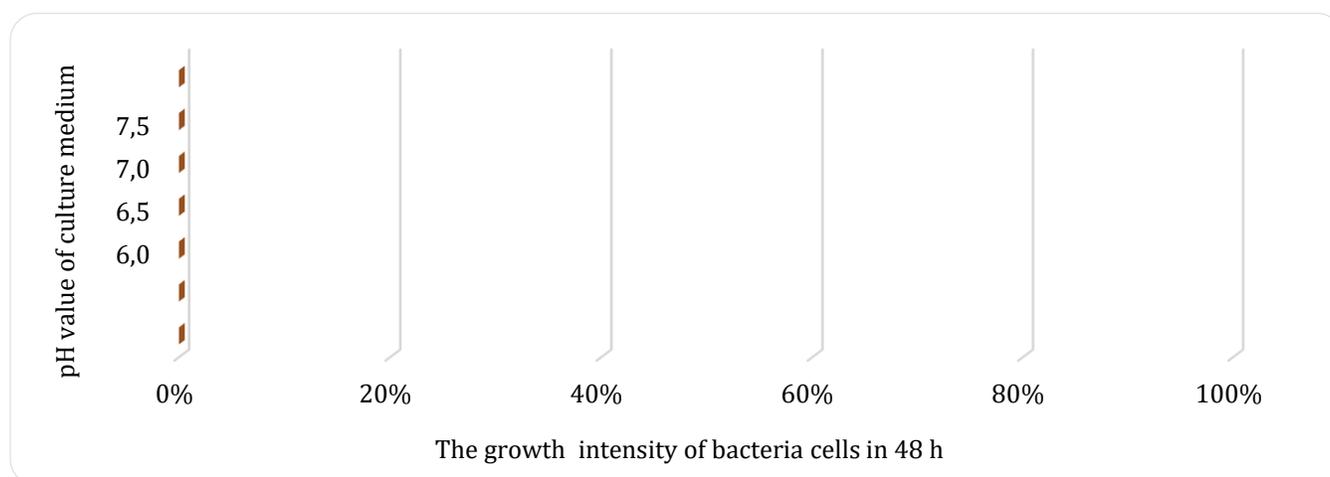


Figure 4: The growth intensity of *Pseudomonas syringae* isolates on media with different pH values.

## The growth *of Pseudomonas syringae* isolates on media at different temperature

All studied isolates were grown in liquid LB medium at temperatures of 15 °C, 20 °C, 27 °C, 35 °C and after 48 hours, the cell titer was determined using a spectrophotometer. Temperature is an important factor directly affecting the virulence of the bacterial canker pathogen. Enzymes of this bacterium involved in pathogenesis are active at a certain temperature regime. As phytopathogens, *Ps. syringae* isolates were able to grow well at 15 °C temperature, in particular, BT-5 and A2.2 isolates (Figure 5). The ability to survive at relatively low temperatures provides the prevalence of potentially pathogenic strains of *Ps. syringae* in non-plant substrates such as irrigation waters or in symptomless weeds, wild plants and ground covers (Morris *et al.*, 2019).

## The sensitivity of *Pseudomonas syringae* isolates to antibiotics

Determining the sensitivity of bacteria to antibiotic substances gives rise to the treatment of diseased plants from bacterial infections. In the appointment, effective treatment is necessary to select antibiotics that can actually suppress the pathogen. For this, first, in laboratory conditions, it is necessary to determine the sensitivity of bacteria to various types of antibiotics.

As the results have shown, all tested *Ps. syringae* isolates

were sensitive to erythromycin, ofltoxacin, and penicillin at least in the 50 mg dose; the lysis zones around the wells made up 12 – 22 ± 0,02 mm (Supplem. material, Figure 2).The sensitivity of the of the bacteria to antibiotics appeared at different times, for example, after two or three days. In our studies, almost all bacterial strains showed sensitivity to antibiotics, forming 10–20 ± 0, 01 mm lysis zones after two days.

In particular, the bacteria turned out to be medium-sensitive, since bacterial colonies continued to grow after some time. The determination of the sensitivity of *Pseudomonas syringae* isolates to antibiotics in laboratory experiments is the first stage to search for non-chemical agents to combat the disease.

## Artificial infection of mature pear fruits to determine the *Pseudomonas syringae* virulence

Pathogenic bacteria use a variety way to infect and multiply in host cells. For example, it is well known that *Erwinia amylovora* cells can produce mucous exudate in immature pear fruits, when *P. syringae* cells can form a dry dark necrosis in fruits. Through the White method (Johansson *et al.*, 2015), the mature pear fruits were infected with *P. syringae* culture. According to the results of these tests, *P. syringae* local strains isolated from apricots, peaches, cherry and plums showed a clear symptom of necrosis in

mature fruits. Within one week, brown necrotic spots appeared in pear fruits, which is a basic symptom of bacterial cancer. Initially, there were brown and eventually black color spots covering the fruits (Figure 6a). This indicated that *P. syringae* isolates with virulence properties could produce compounds such as siderophore pyoverdine and phytotoxin, similar to syringomycin, which cause dark dry decay (Jones *et al.*, 2007; Taguchi *et al.*, 2009).

**Artificial infection of immature cherry and plum fruits to determine the *Pseudomonas syringae* virulence**
The new spring season immature cherry and apricot

fruits of different cultivars were selected for artificial infection tests. As shown in Figure 6b and c, the typical symptoms of bacterial cancer appeared in fruits and brown spots formed around the inoculated points; on the other hand, where sterile water was injected in control, there were no signs of spots. Particularly, the strains S2-3, Sh –ch, and A 2.2 showed clearly visible spots on the surface of cherry fruits, whereas all tested strains demonstrated brown-colored spots on the surface of plum fruits. These results confirm that the detected local P. syringae isolates can cause bacterial cancer symptoms. The fruits inoculated with sterile water in control experiments presented in Figure 7.
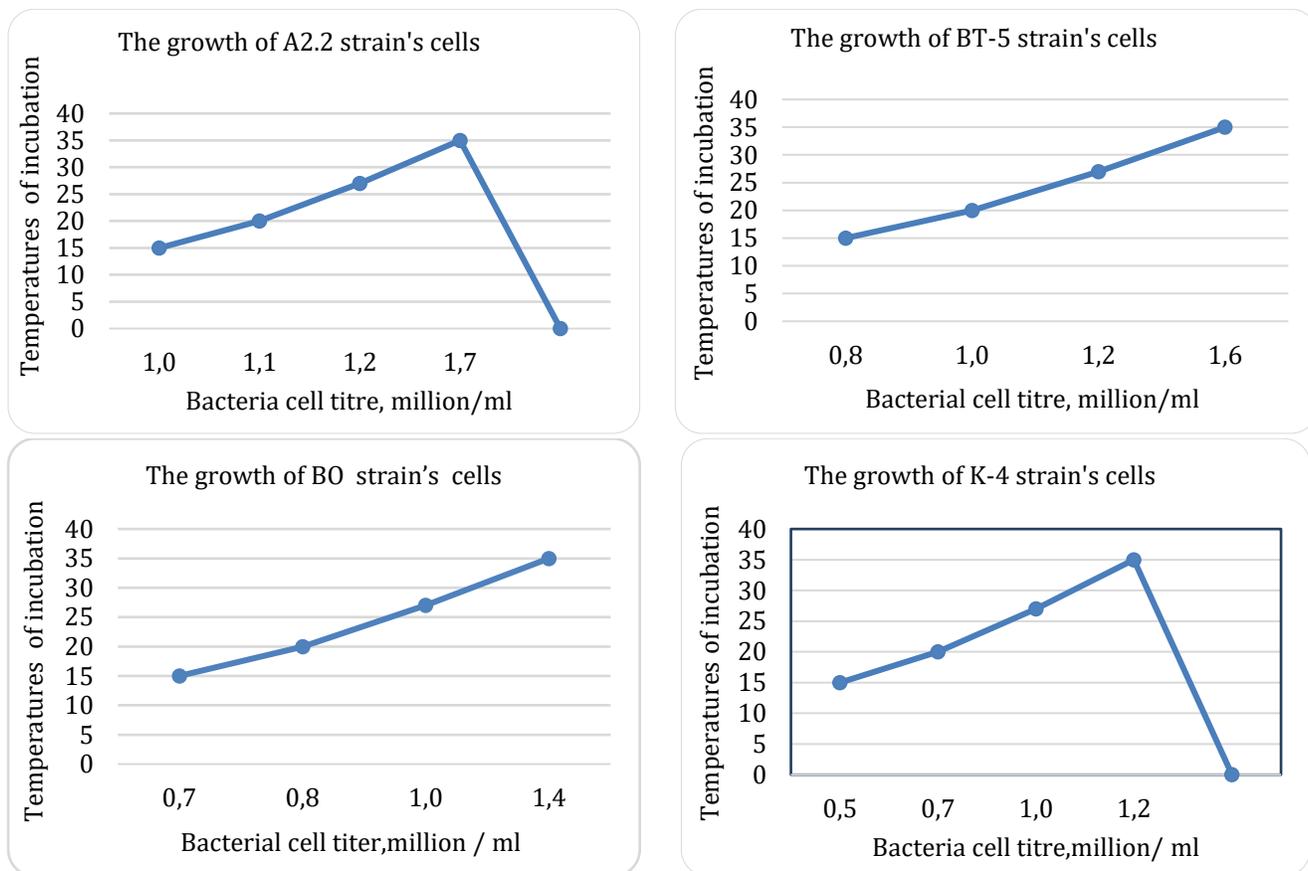


Figure 5. The growth intensity of *Ps. syringae* isolates at different temperatures in liquid LB medium.
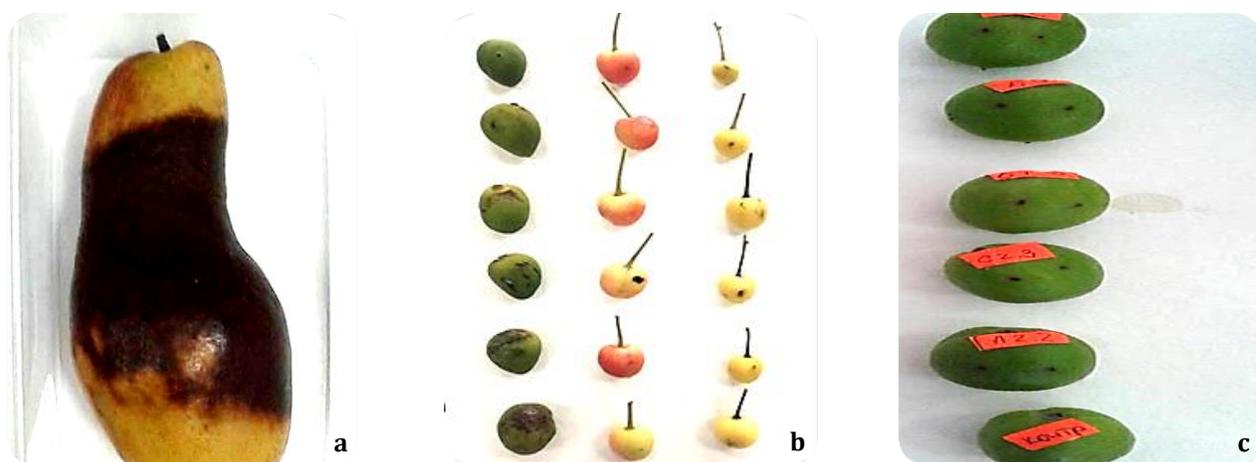
Figure 6: *Pseudomonas syringae  BA*  strain isolated  from Batken Apricot have caused the  necrosis in mature  pear fruits (a);  b and c- the plum, cherry and apricots fruits in artificial infect biotests.
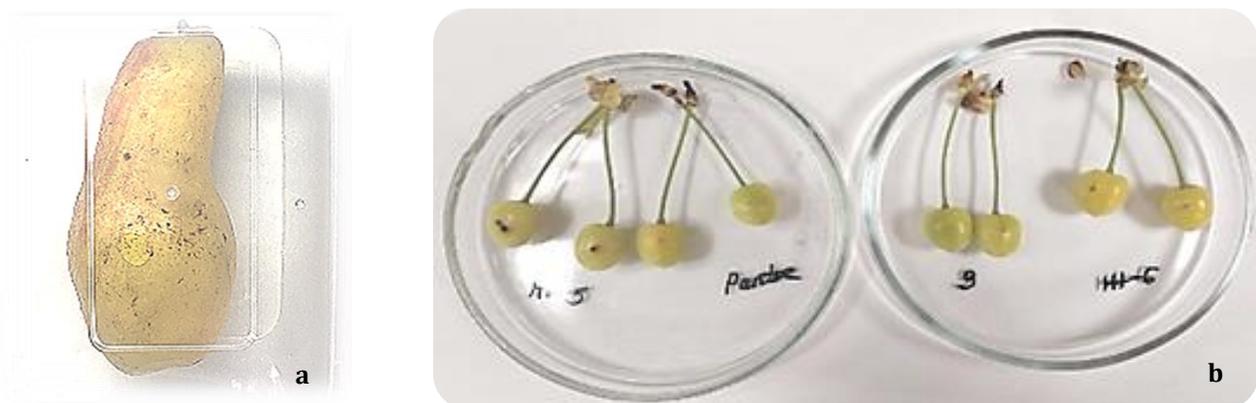


Figure 7: Mature pear fruit in control, inoculated with sterile water ( a); cherry fruits in control, inoculated with sterile water (b).

**The hypersensitivity of plant tissue to *Pseudomonas syringae***

The necrosis patches on surface of the tobacco leaves appeared within 24 hours. The most obtained *Pseudomonas syringae* isolates induced rapid HR and death (within 12–24 h) of tobacco leaf cells at the site of pathogen invasion. The induction of HR requires live and metabolically active bacteria as well as physical contact between plant and bacterial cell walls. According to Figure 3 (Supplemen. material. Figure 3), the BT-5 strain (isolated from Balykchy place) has demonstrated the most visible hypersensitivity reaction of tobacco leaves. Subsequently, a more active reaction of tobacco leaves was caused by Sh-4 (isolated from the peaches) and C2-3 (isolated from the plums).

**Classic analysis based on the polymerase chain reaction**

Molecular methods are currently the most widely adapted and considered very useful for the identification of bacterial canker causal agents and studying their genetic diversity. 16S rRNA gene analyses has found the association of epiphytic microflora consisting except of this pathogen bacteria, gram-negative bacteria from *Stenotrophomonas, Xanthomonas, Erwinia*, genera, and gram positive bacteria from the *Bacillus* genus (Figure 8).

**The determination of the antagonistic effects of Streptomyces against *Pseudomonas syringae***

As well known among the actinomycetes, the representatives of the *Streptomyces* genus are important, as they include substances with antagonistic properties against bacterial and fungal pathogens in addition to a bio-stimulating effect on seeds and seedlings of agricultural plants (Gupta *et al.*, 1995; Kolla and Vijayalakshmi, 2009).

In this study, *Streptomyces* active strains from the laboratory collection were used for biotesting against

this pathogen. The origin and cultural characteristics of the antagonists are shown in (Supplem. material, Table 1). The results obtained when using the touch method of the nutrient medium surface are shown in Table 5.

As the results of the research have shown, the antagonistic activity of different biocontrol strains was different. The strain *Streptomyces* bambargiensis SK-6.6 showed a strong antagonistic effect on *P. syringae* providing a complete inhibition of the growth and development of the colonies of this phytopathogen. Other biocontrol agents such as *Streptomyces* sp. C1-4 and *Streptomyces* sp. Pr-3 showed a hyper-parasitic effect, using the colonies of *P. syringae* as a source of nutrients, growing on the cells of pathogens.

### Screening of *Streptomyces strains on* apricot and black plum seedlings

Five days after artificial infestation of the highly susceptible local variety of apricot and black plum seedlings the first signs of the disease's symptoms emerged: the leaf first appeared water-soaked; they next turned dark green, then wilted and finally turned

brownish to black (Supplem. material, Figure 5). Seven days after infection, the seedlings' leaves were treated with *Streptomyces C1-4* at a dose of $10^6$ spores/ml. A second treatment with the antagonist product was conducted seven days after the first treatment, using the same dose. 15 days after two applications of the antagonist product with a dose of $10^6$ spores/ml, the number of recovered leaves has increased; the number of apricot healthy leaves reached 33±0.03 (Figure 9).

On 15 days after two treatments with *Streptomyces sp. C1-4*, the number of infected leaves had not increased and progression of the disease had completely stopped, a diseased plant's tissue can begin to recover and function normally. When a biological product based on *Streptomyces sp. C1-4* was used, the sick parts dried up and the uninfected parts were preserved in their natural state; the leaf tissue did not become rigid, as with a chemical drug. Thus, the biological antagonist affects only the pathogen and has no adverse effect on leaf tissue. This means that by stopping the progression of the disease, a diseased plant's tissue can begin to recover and function normally.
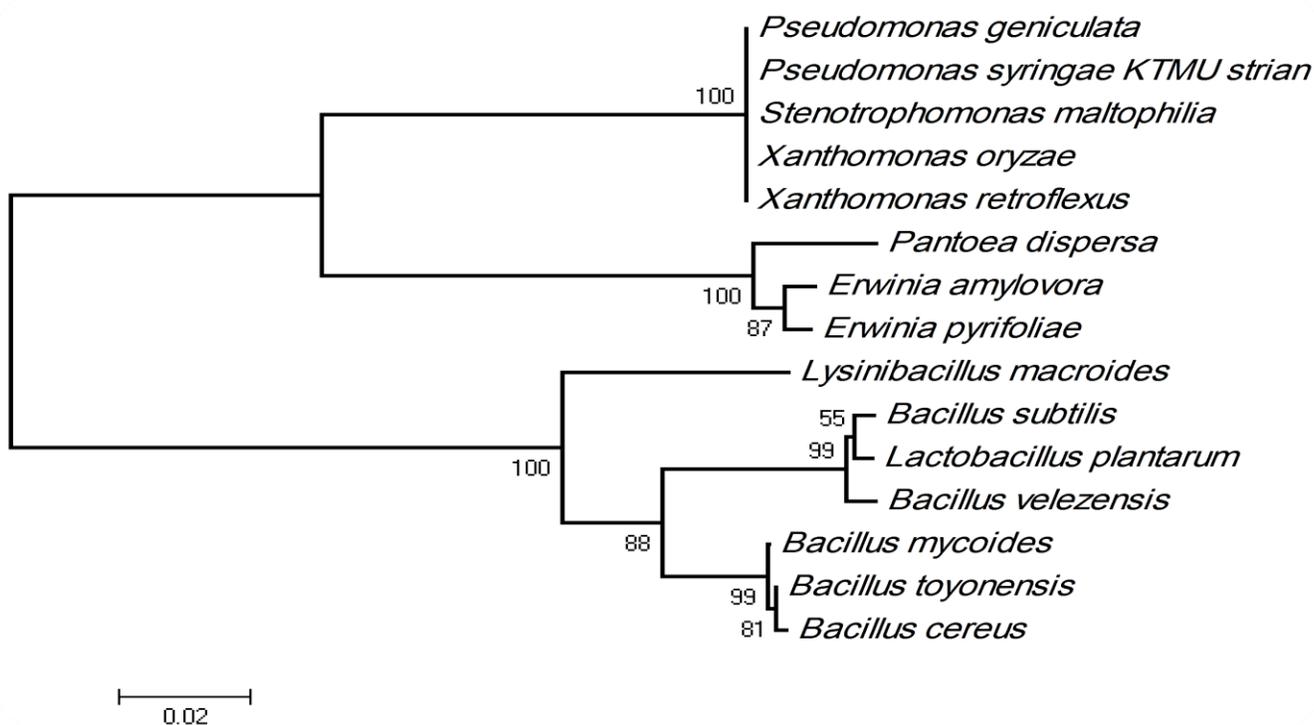


Figure 8: Phylogenetic tree produced from the I6S gene sequences showing the phylogenetic relationships of *P. syringae KTMU strain* with gram negative and gram-positive bacteria using the neighbor-joining method. Relevant bootstrap values (expressed as a percentage of 1000 replicates) are shown at branch points, and bar (0.02) changes per sequence position.

Table 5: Antagonistic activity of *Streptomyces* species  against bacterial blight pathogen in laboratory experiments (n=3).

| Test culture | Antagonistic activity of the microorganisms after seven days | | | | | |
| | *Streptomyces diastochromogenes, Sk-6* | *Streptomyces* sp. *SK-2, 2* | *Streptomyces* sp. *KK-4* | *Streptomyces* sp. *C1-4* | *Streptomyces* sp. *K-3.3* | *Streptomyces* sp. *Pr-3* |
|---|---|---|---|---|---|---|
| *P. syringae* | Inhibits growth of pathogen | Inhibits growth of pathogen | Shows no activity | Hyper parasitism | Shows no activity | Hyper parasitism |
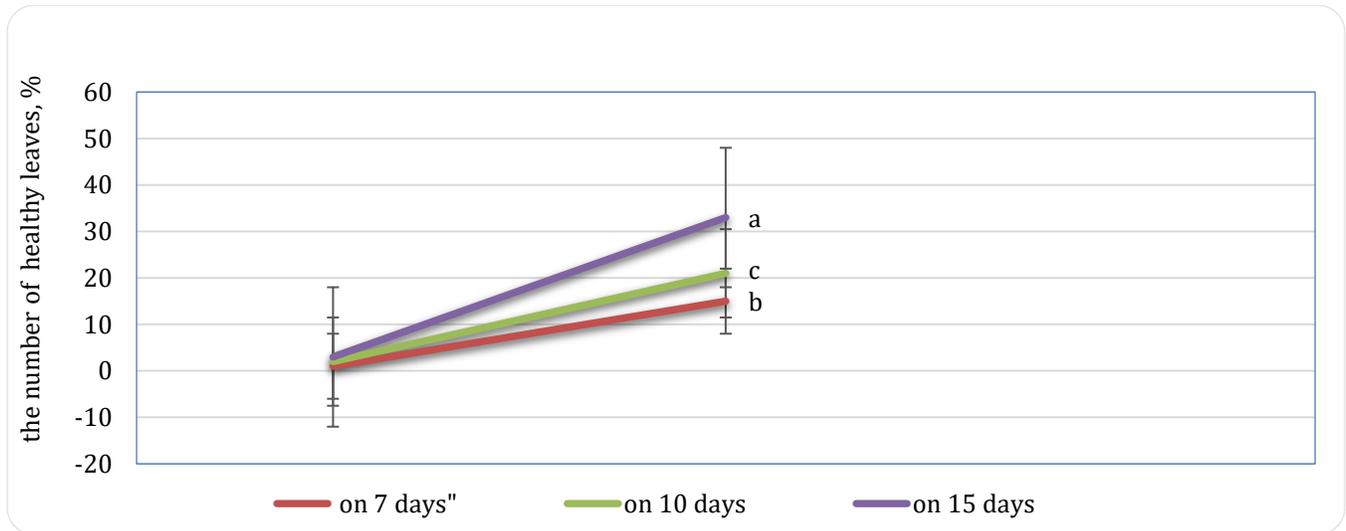


Figure 9: The increasing dynamic of healthy seedling's leaves in 15 days after two applications of Streptomyces C1-4 at a dose of $10^6$ spores / ml;  Values are means of ± SD, n=3, P≤0.05.
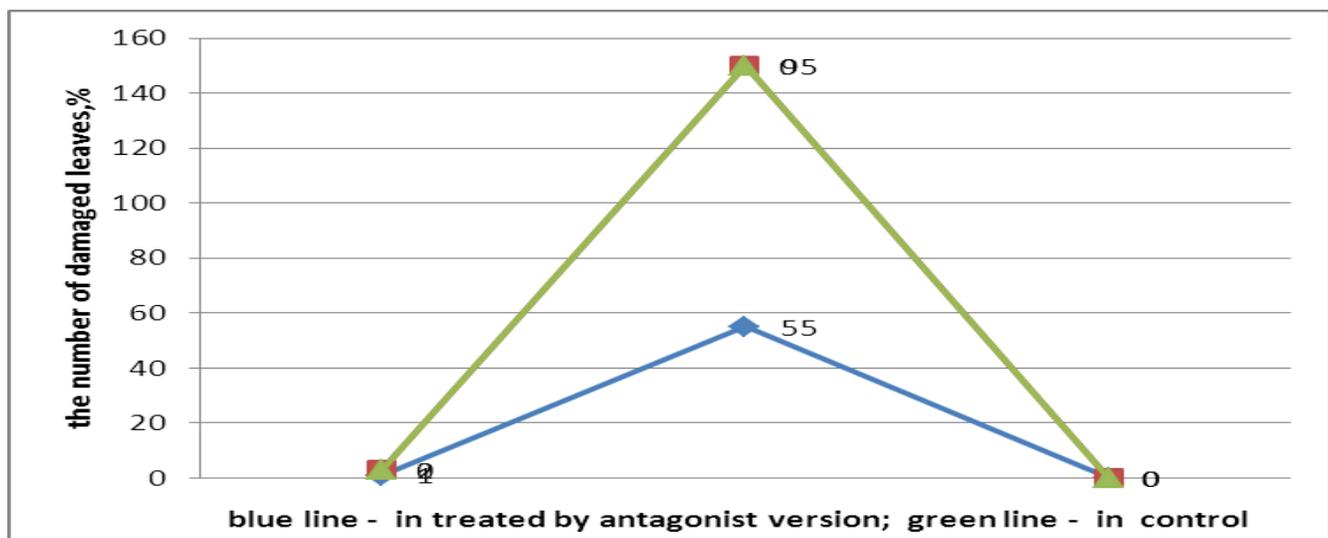


Figure 10: The decreasing dynamic of damaged seedling's leaves in 15 days after two applications of Streptomyces C1-4 at a dose of $10^6$ spores/ml. Values are means of ± SD, n=3, P≤0.05.

## DISCUSSION

*P. syringae* as an economically important pathogen remains largely unexplored and is far from properly understood in Kyrgyzstan. A lot is still unknown about the biology, host range, and ecological behavior of these bacteria on their host plants in the different ecosystems of the country. The present study was conducted for the first time in Kyrgyzstan's conditions for the study of the

biology, ecology, and the prevalence of the pathogen in different regions with different climatic conditions. The monitoring survey for the symptomatic observation and collection of samples has revealed that in old stone plants planted over 30–40 years ago, the characteristic symptoms for bacterial cancer were strongly exhibited with large ulcer wounds and necrotic bark formations with the release of the exudate amber formation on the trunks and branches. The prolonged coexistence of the pathogen and the host under certain climatic conditions apparently could provide to both sides a survival status, but with a gradual weakening of host plant. The used classical biochemical tests (LOPAT and GATTa's) have allowed us to isolate pure cultures of pathogen bacterium and identify the obtained isolates as a pathogen—*P. syringae*. Additionally, 16Sr RNA gene analysis has found the association of epiphytic microflora consisting except this pathogen bacteria, gram-negative bacteria from *Erwinia, Xanthomanas, Stenotrophomonas* genera, and gram-positive bacteria from the *Bacillus* genus. In such an association, this pathogen in a dominant ratio has adapted to coexist with its host plant and associated epiphytic bacteria in different climatic zones of the country. In the composition of the epiphytic microflora after the pathogen, the bacteria of the *Stenotrophomonas* genus was dominated. The role of this bacterium as a saprophyte or facilitative parasite contributing to the development of an outbreak must be clarified in future studies. According to the scientists, these bacteria can be found throughout the environment, particularly in close association with plants. *S. maltophilia* frequently co-occurs and forms multispecies biofilms with *P. aeruginosa* (Ryan *et al.*, 2009). As reported by studies (Ercolani, 1991, 1985), that many phyllospheric bacteria are non-spore-forming, gram-negative or gram-positive heterotrophs, in general, the same species or group of bacteria are present on a wide range of plants, which was confirmed in our results. In addition, the studies have concluded that these groups are well adapted to the life in the phyllosphere, the conditions of which change daily, seasonally, while the pathogens on the diseased plants with obvious symptoms     are predominated in such communities (Hirano and Upper, 2000). Our results also approved the above reports.

The long coexistence of the pathogen-plant system in the plant's homeland is a prime example for this bacterium since we found such a system between apricot and this bacterium in Batken's homeland, between peach varieties and this bacterium, between plum, cherry varieties and this bacterium in other regions; almost every local stone fruit variety had pathogen such as *P. syringae*. The composition of epiphytic microflora did not differ much in the regions of the republic, and no large differences were observed in the seasons of the year.

The dominance of *P. syringae* in all climatic conditions and seasons of the year indicates a wide range of the temperature of its growth. The optimum temperature turned out to be 28 ℃; at this temperature, the isolates isolated from apricot, peach, cherry and plum in just two to three hours, which gave a good growth of colonies on media. At a temperature of 15 ℃, they had intensive growth, which indicates that at this temperature, they do not lose their virulence. Another adaptive nature inherent in the isolated isolates is their ability to grow at different pH values, ranging from 6.0 to 7.5. Therefore, they affect plants in all phases of development, from budding to ripe fruit, as well as all age stages, spanning from seedlings to old, perennial trees. The results of biotests on mature and immature fruits of different stone fruits proved this ability. Unlike the *Erwinia* bacteria, this pathogen causes dry rot in the fruit. The presence of pectolytic enzymes detected by culturing them on media containing hawthorn and currant extracts proves that this pathogen is capable of destroying plant tissues in case of damage.

As well known, the causative agent of bacterial canker *P. syringae* bacteria affects more than 180 species of plants, among them herbaceous plants, vegetables, fruit crops. To limit the use of chemicals to protect plants from bacteriosis caused by *P. syringae* a constant search of safely to the environment and human health alternative means is underway.

For this purpose, the antibacterial activity of the growth-stimulating rhizospheric bacteria (PGPR) such as *P. aeruginosa, Bacillus stratosphericus, Paenibacillus polymyxa, B. subtilis*  and others against *P. syringae* was studied *in vitro* and *in vivo* experiments. Their overwhelming disease activity against *P. syringae* was reliably proven on tomatoes, kiwi fruit trees (Hong *et al.*, 2016; Donati *et al.*, 2018).

The biocontrol potential of soil and rhizospheric actinomycetes like *Streptomyces* in the regulation of pest damage by *Pseudomonas syringae* in fruit trees remains almost   unexplored.   According   to   studies,   plant-

associated actinomycetes play an important role in plant health due to ability to produce a wide range of secondary metabolites, like siderophores, various plant phytohormones such as auxins, cytokinins and gibberellins, and antibacterial and antifungal compounds (Cassán *et al.*, 2001; Bottini *et al.*, 2004; Solans *et al.*, 2011). It has been proven that *Streptomyces* species are capable of exerting antifungal and antibacterial properties, protecting the plant root system from pathogens, and also suppressing diseases in soils (Gopalakrishnan *et al.*, 2011; Xiao *et al.*, 2002; Mendes *et al.*, 2011; Cha *et al.*, 2015).

In contrast to studies where *Streptomycetes* were used by introducing into the soil and around the root system of diseased plants, we used the *Streptomyces* strains against the bacterial canker pathogen by spraying the leaf surface and the crown of diseased seedlings of stone fruits, so biocontrol agents were introduced into epiphytic plant associations.

When signs of the disease appear on the leaves, double plentiful spraying of affected plants with a suspension of *Streptomyces* C1-4 strain ($10^6$ spores/ml) with an interval of 7 days prevented the development of the disease. Fifteen days after the biologic agent was applied twice, the plants fully recovered, new leaves and new twigs appeared. These results indicate that the *Streptomyces* C1-4 strain, introduced as a biological agent into the phyllosphere of diseased plants was able not only to colonize the epiphytic microflora, having an antibacterial effect but also increased the protective ability of the plants themselves, their potential to resist the disease, which is consistent with the data of other researchers (Conn *et al.*, 2008; Tarkka *et al.*, 2008; Kurth *et al.*, 2014), they noted that *Streptomycetes* can cause a state of the increased protective ability of plants against pathogens, thereby providing plants with induced systemic resistance.

The growth of new shoots in a short period and the complete restoration of damaged leaves, branches also indicates to the growth-stimulating or phytohormonal effect of this agent on plants, as noted in the studies of (Cassán *et al.*, 2001; Bottini *et al.*, 2004). Thus*, Streptomyces* strain C1-4 was selected after *in vitro* tests as an effective antagonist and hyper-parasitic agent to *P. syringae* for spraying the diseased leaves of apricot and black plum seedlings. *Streptomyces C1-4* showed a significant effect against *P. syringae* after two applications. A suitable time for this bio product's application is spring, when the symptoms of bacterial canker start to develop on the leaves of trees and the air temperature is 18–23 °C.

## CONCLUSIONS
Therefore, our results on diagnosis of this pathogen can provide practical information useful for implementing efficient control measures in the current cropping season in the stone fruit gardens and in limiting future disease outbreaks of bacterial cancer in different regions of country. Also obtained results about capacity of the pathogen to adapt to climatic conditions, sensitivity to such biocontrol agents as *Streptomyces* species can encourage to develop a local bio-product based on this bioagent for spraying stone fruits with the initial manifestation of disease symptoms and to conduct preventive treatments in the fall and spring to increase the plant's resistance to pathogens. Our studies are suggesting the creation of induced plant immunity by spraying the phyllosphere with biological agents.

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**CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

**AUTHORS CONTRIBUTIONS**

All the authors contributed equally to this work.

**SUPPLEMENTARY MATERIALS**
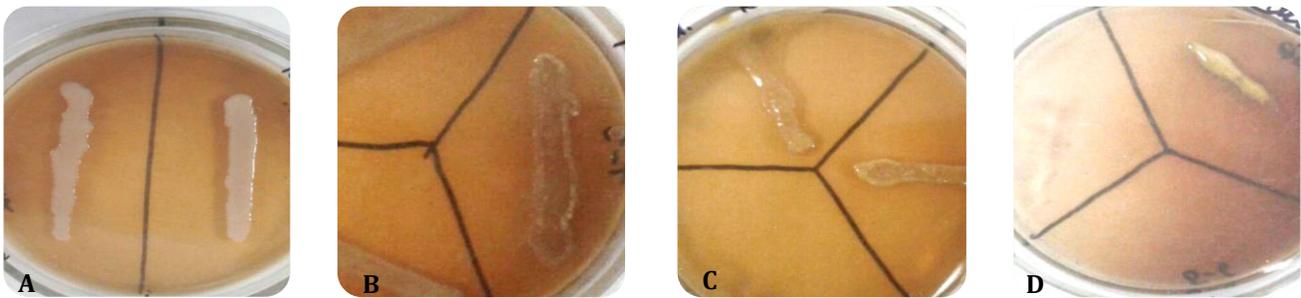


Supplementary Figure 1. The colonies of pectate degrading P. *syringae* isolates on a pectin-containing medium: A, B and C - with hawthorn containing agar; D- with currant containing agar
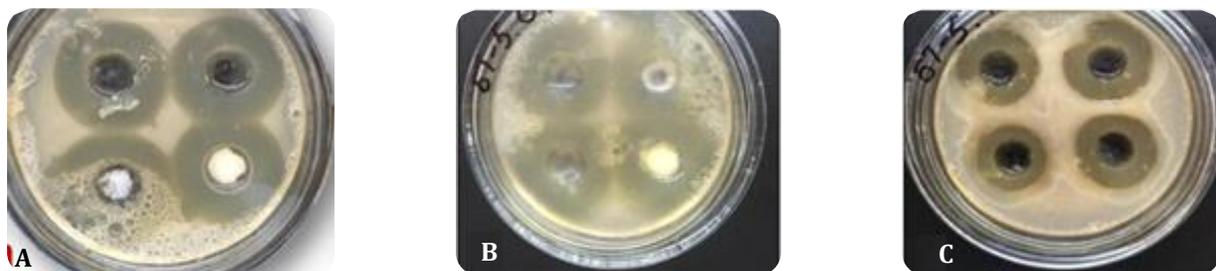


Supplementary Figure 2. The sensitivity of Pseudomonas *syringae* isolates to antibiotics (A-erythromycin; B-ofloxacin; C-penicillin).

Supplementary Figure 3. Hypersensitivity reaction in tobacco leaves.



Supplementary Figure 4. A) In 24 hours  and  B)  5 days after infection  apricot seedlings with diseased symptoms on the leaves by  spraying with  *Pseudomonas syringae*  (1x 106 ml$^{-1}$).



Supplementary Figure 5. Recovering leaves of apricot  and black plum seedlings 30 days after second treatment by *Streptomyces C1-4* at a dose of $10^6$ spores/ml.

Supplementary Table 1. The origin and cultural features of *Streptomyces* strains used in this study.

| Name of strains | Site of isolation | Cultural characteristics on SAA (starch ammonium agar) |
|---|---|---|
| *Streptomyces fumanus, gn-2* | From the rhizosphere of mustard (*Sinapis alba*), the Issyk-Kul region | It forms velvety aerial mycelium of a pink-lilac colour and with a specific odour on SAA |
| *Streptomyces diastochromogenes, Sk-6.6* | From soil of Son-Kul Valley, at an altitude of 3200 m above sea level | It forms an aerial mycelium with a white-yellowish colour and a specific odour on SAA |
| *Streptomyces sp. C1-4* | From soil of Son-Kul Valley, at an altitude of 3200 m above sea level | It forms an aerial mycelium with a light-green colour and specific odour on SAA |
| *Streptomyces sp. Pr-3* | From soil of experimental field of Agricultural Faculty, KTU Manas | It forms an aerial mycelium with a lemon-yellow colour, white edge; the surface is raised on SAA |
| *Streptomyces sp. KK-4* | From soil of experimental field of Agricultural Faculty, KTU Manas | Forms aerial mycelium of a yellow colour on SAA, substrate mycelium is dark red. The colonies form a continuous growth on the surface of the medium |
| *Streptomyces sp. K-3.3* | From soil of experimental field of Agricultural Faculty, KTU Manas | Forms aerial mycelium of a white colour on SAA |
| *Streptomyces sp. ПАТ-3* | From soil of experimental field of Agricultural Faculty, KTU Manas | Forms aerial mycelium of a white colour on SAA, substrate mycelium is pink in colour |