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## ИЗВЕСТИЯ

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РЕСПУБЛИКИ КАЗАХСТАН  
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## NEWS

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of the Institute of Plant Biology and Biotechnology

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**СЕРИЯ**

**БИОЛОГИЧЕСКАЯ И МЕДИЦИНСКАЯ**



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## THE STRUCTURE OF THE MICROBIAL CENOSIS OF PHYLLOSPHERE OF FRUIT CROPS AFFECTED BY FIRE BLIGHT IN SOUTHERN KAZAKHSTAN

**Abstract.** The structure and diversity of microbial cenosis of the apple and pear phyllosphere in the zones of industrial horticulture of Kazakhstan, where fruit trees were affected by fire blight, was studied. The study was carried out in Zhambyl, South Kazakhstan and Almaty regions. 47 samples (shoots, leaves, fruits) from the phyllosphere of healthy and infected organs of fruit trees were collected. In total 216 microbial isolates were selected. Taxonomic identification of microbial species based on cultural and morphological traits was carried out. The isolates were grouped into the following genera: bacteria (142 isolates): *Erwinia*, *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Paenibacillus*; fungi (37 isolates): *Monilia*, *Venturium*, *Aspergillus*, *Podosphaera*, *Penicillium*, *Fusarium*, *Triothecium*, *Mucor*, *Verticillium*, *Alternaria*; yeast and yeast-like microorganisms (37 isolates): *Cryptococcus*, *Rhodotorula*, *Pullularia*, *Saccharomyces*. Bacteria have been found to predominate in the microbiocenosis. In all samples infected with fire blight, bacteria of the genera *Erwinia* and *Pseudomonas* were detected.

Screening of potential bacterial antagonists to *E. amylovora* showed that some of the *Bacillus*, *Pseudomonas* and *Paenibacillus* species have demonstrated inhibitory effect. The diameter of inhibition zones have been ranged from 9 mm to 48 mm. *Bacillus amyloliquefaciens* demonstrated maximal antagonistic activity (48 mm).

**Keywords:** microbial cenosis, *Erwinia amylovora*, fire blight, bacteria-antagonists, biocontrol.

**Introduction.** Fire blight is one of the most harmful diseases caused by gram-negative bacteria *Erwinia amylovora*, affecting several species of the *Rosaceae* family. This disease is found in most of the world's regions and can cause significant losses in important fruit crops, such as apple and pear [1]. Orchards protection from this disease is associated with the implementation of fire blight management. Strategy to increase the fitness of a biological control agent upon delivery to the field is the use of copper-based preparations or antibiotics in combination with agrotechnical techniques [2]. However, formulations containing copper are not effective enough, and the use of antibiotics, which are also used for human and animals' treatment, is banned in many countries [3-5]. Such requirements derive from the risk of positive selection of antibiotic resistant strains of *E. amylovora* and the negative impact on the environment [5-7]. For this purpose, biological control of fire blight using microbial antagonists is considered as alternative to chemical control.

Associated with fruit trees, epiphytic microorganisms play an important role in their development, participating in the supply of plant nutrients, phytohormones, vitamins and other growth factors. Many of these microorganisms produce compounds that inhibit pathogenic microflora (toxins, antibiotics, siderophores), and also contribute to the appearance of induced resistance to phytopathogens in partner plants [3]. In this regard, the identification of new wild isolates from the phyllosphere of plants with inhibitory effect against the fire blight *E. amylovora* pathogen, adapted to local climatic and ecological conditions, as well as the biological traits of the pathogen, are of particular relevance. Such type of novel

strains has potential for creation on its basis complex biopreparations with fire blight controlling and phyto regulatory properties.

The objectives of this work were to study the structure of microbial cenosis of the phyllosphere of fruit crops infected by fire blight in southern Kazakhstan and to identify novel wild bacterial antagonists for the biocontrol of fire blight disease.

We have shown in our previous studies that non-pathogenic strain *Streptomyces canofumeus* is effective against *E. amylovora* [8, 9].

**Materials and methods.** Samples of infected and healthy apple and pear organs (shoots, leaves, fruits) selected from the industrial horticulture areas of Zhambyl, South Kazakhstan and Almaty regions of Kazakhstan.

The samples were collected in compliance with the rules of transportation of biological material, excluding external contamination and ensuring the safety of the original microflora of the samples [10]. The microbial community structure and diversity of the apple and pear phyllosphere, phenotypic and morphological traits such as colony color and cell motility as well as physiological fingerprints of microbial isolates were performed using conventional bacteriological methods [11-13]. The isolates were identified according to Satton D. et al. and Bergey's Manual of Systemic Bacteriology [14, 15]. Gram staining was performed as previously described by Claus [16].

All isolates were examined for their ability to inhibit the growth of *E. amylovora* by agar-diffusion-test [17]. This test was repeated with three replicates and the diameter of inhibition zones was assessed.

**Results and discussion.** 47 samples of infected and healthy organs of apple and pear plants from orchard cenosis of Zhambyl, South Kazakhstan and Almaty regions, were selected.

The studies of the structure of microbiocenosis of the apple and pear phyllosphere in Southern Kazakhstan revealed that bacteria (142 isolates) were numerically dominated. The number of other groups of microorganisms was lower: fungi – 37 isolates and yeast – 37 isolates. The structure and diversity of epiphytic microorganism's community in the phyllosphere of fruit crops in the observed orchard microbiocenosis is presented in tables 1 and 2.

Taxonomic identification of isolated pathogenic and concomitant microflora by conventional bacteriological methods has demonstrated that the structure of the examined microbiocenosis was represented by the following taxonomic groups.

Table 1 – The structure of epiphytic microorganism's community isolated from the affected apple and pear trees in Southern Kazakhstan

| Region                  | Number of samples, pcs | Number of isolates, pcs. |          |       |       |
|-------------------------|------------------------|--------------------------|----------|-------|-------|
|                         |                        | Total                    | Bacteria | Fungi | Yeast |
| Zhambyl oblast          |                        |                          |          |       |       |
| Zhambyl                 | 8                      | 42                       | 30       | 5     | 7     |
| Baizak                  | 10                     | 23                       | 13       | 6     | 4     |
| South Kazakhstan oblast |                        |                          |          |       |       |
| Sairam                  | 5                      | 38                       | 26       | 7     | 5     |
| Tolebi                  | 7                      | 24                       | 15       | 3     | 6     |
| Almaty oblast           |                        |                          |          |       |       |
| Karasai                 | 6                      | 37                       | 28       | 3     | 6     |
| Talgar                  | 6                      | 33                       | 20       | 8     | 5     |
| Enbekshikazakh          | 5                      | 19                       | 10       | 5     | 4     |
| Total                   | 47                     | 216                      | 142      | 37    | 37    |

Table 2 – The diversity of epiphytic microorganism's community isolated from the infected apple and pear trees in Southern Kazakhstan

| Region                  | Epiphytic microorganisms  |  |   |
|-------------------------|---|--|---|
|                         | Bacteria  | Fungi  | Yeasts  |
| Zhambyl oblast          | <i>Erwinia amylovora</i><br><i>Pseudomonas syringae</i><br><i>Bacillus polymixa</i><br><i>Bacillus amyloliquefaciens</i>  | <i>Monilia fructigena</i><br><i>Trichoderma asperellum</i><br><i>Alternaria sp</i><br><i>Penicillium glaucum</i><br><i>Podosphaera leucotriha</i><br><i>Verticillium attenuatum</i>  | <i>Cryptococcus albidus</i><br><i>Rhodotorula rubra</i><br><i>Pullularia pullulans</i>                                    |
| South Kazakhstan oblast | <i>Pseudomonas syringae</i><br><i>Erwinia amylovora</i><br><i>Bacillus polymixa</i>   | <i>Monilia fructigena</i><br><i>Trichoderma asperellum</i><br><i>Aspergillus sp</i><br><i>Mucor sp</i><br><i>Aspergillus niger</i><br><i>Podosphaera leucotriha</i>  | <i>Cryptococcus albidus</i><br><i>Pullularia pullulans</i><br><i>Saccharomyces cerevisiae</i>                             |
| Almaty oblast           | <i>Lactobacillus plantarum</i><br><i>Erwinia amylovora</i><br><i>Bacillus polymixa</i><br><i>Bacillus amyloliquefaciens</i><br><i>Paenibacillus polymixa</i><br><i>Pseudomonas syringae</i> | <i>Monilia fructigena</i><br><i>Fusarium oxysporum</i><br><i>Trichoderma asperellum</i><br><i>Fusarium oxysporum</i><br><i>Podosphaera sp</i><br><i>Mucor sp</i><br><i>Penicillium glaucum</i><br><i>Aspergillus fumigatus</i> | <i>Cryptococcus albidus</i><br><i>Rhodotorula rubra</i><br><i>Pullularia pullulans</i><br><i>Saccharomyces cerevisiae</i> |

Bacterial community was represented by 5 genera: *Erwinia*, *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Paenibacillus*; fungi by 10 genera: *Monilia*, *Venturiua*, *Aspergillus*, *Penicillium*, *Fusarium*, *Trihothecium*, *Mucor*, *Verticillium*, *Alternaria* (figures 1, 2). The causative agent of powdery mildew – *Podosphaera* fungus, was determined only by microscopic analysis, as far as it is an obligate pathogen, and its morphological and cultural traits and testing for antagonistic activity was not possible to study. Yeasts were represented by 4 genera: *Cryptococcus*, *Rhodotorula*, *Pullularia*, *Saccharomyces*.

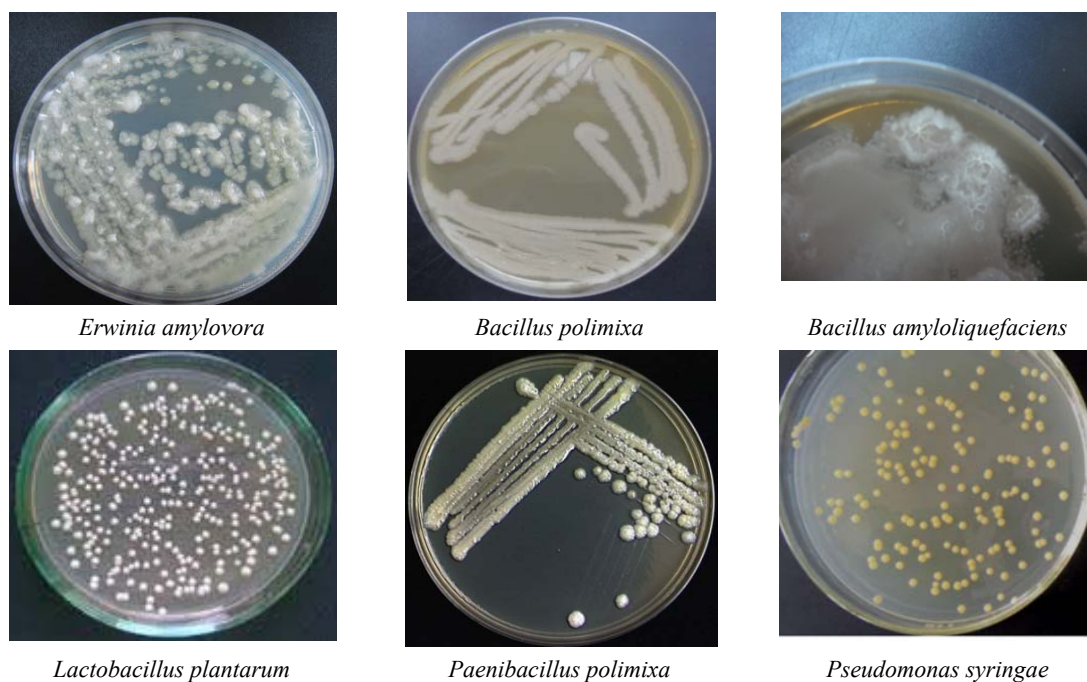


Figure 1 – Colonies of bacteria isolated from the apple and pear phyllosphere



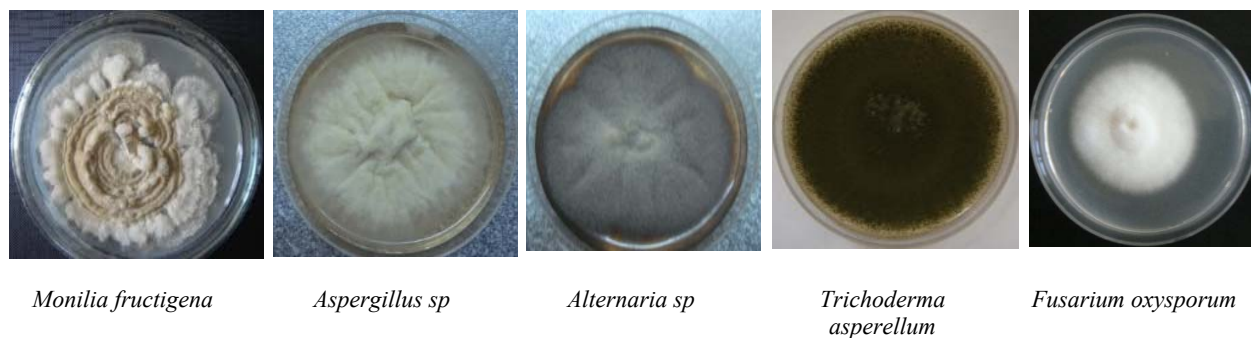


Figure 2 – The main types of fungi isolated from the phyllosphere of apple and pear

Due to the fact that the samples were collected in orchards, where fire blight was previously recorded, *Erwinia amylovora* and *Pseudomonas syringae* were found in all studied samples in close symbiosis (figure 3).

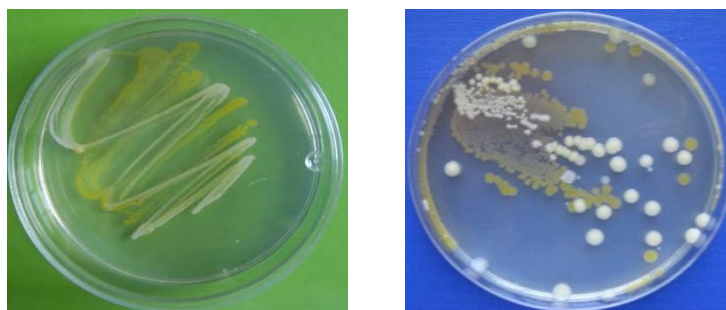


Figure 3 – *Erwinia amylovora* and *Pseudomonas syringae* bacterial colonies on agar plates

Initial screening *in vitro* conditions of epiphytic microorganisms isolated for the ability to inhibit the growth of *E. amylovora* was carried out by agar-diffusion-test. The results of the screening showed that some species of *Bacillus*, *Pseudomonas* and *Paenibacillus* genera demonstrated antagonistic activity against *E. amylovora*. The diameters of inhibition zone have been depended on the species and ranged from 9 mm to 48 mm. The maximum diameter of inhibition zone (48 mm) was exhibited by *B. amyloliquefaciens* (figure 4).

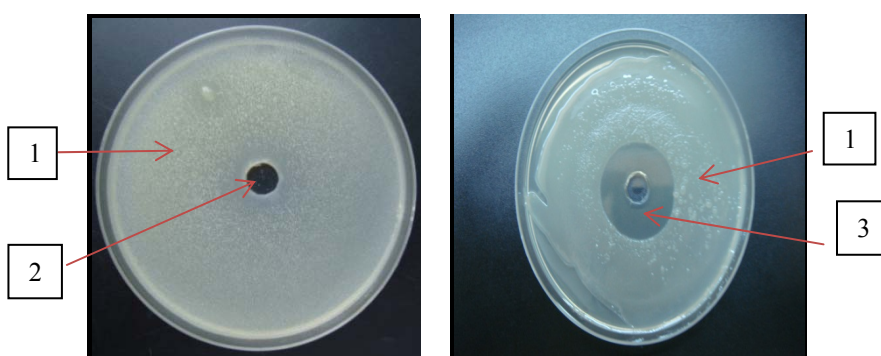


Figure 4 – Inhibition of the *E. amylovora* growth:  
1- *E. amylovora*, 2 - sterile water, 3 - *B. amyloliquefaciens*

The studied strains of fungi and yeasts have not exhibited antibacterial activity towards *E. amylovora*.

Thus, as a result of the present study it was found that epiphytic microflora of orchard's cenosis of Zhambyl, South Kazakhstan and Almaty regions of Kazakhstan is characterized by a large variability,



both in structure and diversity. In all collected samples *E. amylovora* was isolated in close symbiosis with *P. syringae*, which is the causative agent of bark necrosis. Symptoms of diseases caused by *P. syringae* are similar to fire blight, although there is a difference – in case of bark necrosis there are no "hooks" on young shoots typical for fire blight [18].

The efficacy of novel bacterial strains isolated from the apple and pear phyllosphere in the zones of industrial horticulture of the South of Kazakhstan to control *Erwinia amylovora* was evaluated under *in vitro* conditions. *B. amyloliquefaciens* MV<sub>40</sub> demonstrated high antagonistic activity against the pathogen in agar-diffusion-test. The efficacy of epiphytic bacteria *B. amyloliquefaciens* FZB42, *B. amyloliquefaciens* LMR2 against fire blight has also been reported by Chen et al. (2009) and Ait Bahadou et al. (2018) [19, 20]. It was shown that the antagonistic activity of *B. amyloliquefaciens* FZB42 strain may be due to the production of polyketide of dipyrindine and dipeptide bacilysin compounds [19].

In our further studies, it is planned to study active metabolites with inhibitory effect to *E. amylovora*. The novel strain *B. amyloliquefaciens* MB40 may be considered as potential candidates for development microbial formulation efficient against fire blight disease.

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### **ҚАЗАҚСТАННЫҢ ОҢТҮСТІК АЙМАҚТАРЫНДА БАКТЕРИЯЛЫҚ КҮЙІКПЕН ЗАҚЫМДАЛҒАН ЖЕМІС ДАҚЫЛДАРЫНЫҢ ФИЛЛОСФЕРАСЫНЫҢ МИКРОБТЫҚ ЦЕНОЗЫНЫҢ ҚҰРЫЛЫМЫ**

**Аннотация.** Бұрын жеміс ағаштары бактериялық күйікпен зардап шеккен, Қазақстанның өнеркәсіптік бау-бақша шаруашылығы аймағында алма және алмұрт филлосферасының микробтық ценозының құрылымы мен алуан түрлілігі зерттелді. Зерттеу жұмыстары Жамбыл, Оңтүстік Қазақстан және Алматы облыстарында жүргізілді. Жеміс ағаштарының сау және зақымдалған мүшелерінің филлосферасынан 47 үлгі (өркендер, жапырақтар, жемістер) алынды. Олардан жалпы 216 микробтық изоляттар бөлініп алынды. Дақылды-морфология сипаттамасы негізінде микроағзалар дақылдарының таксономиялық идентификациясы зерттелді. Изоляттар келесі туыстарға топталған: бактериялар (142 изоляттардан құралған): *Erwinia*, *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Paenibacillus*; саңырауқұлақтар (37 изоляттардан құралған): *Monilia*, *Venturium*, *Aspergillus*, *Podosphaera*, *Penicillium*, *Fusarium*, *Triothecium*, *Mucor*, *Verticillium*, *Alternaria*; ашытқы және ашытқы тәрізді микроағзалар (37 изоляттардан құралған): *Cryptococcus*, *Rhodotorula*, *Pullularia*, *Saccharomyces*. Микробценоздың құрамында ең басым болғаны бактериялар. Барлық зақымдалған мүшелерден бөліп алынған үлгілерді зерттегенде *Erwinia* және *Pseudomonas* туысына жататын бактериялар кездесетіні анықталды.

*Bacillus*, *Pseudomonas* және *Paenibacillus* туысының өкілдерін *E. amylovora* қарсы антагонисттік белсенділіктерін тексеруге жүргізілген скрининг нәтижелері көрсеткендей, жекелеген түрлерге байланысты патогеннің өсуін шектеуге қабілеттілігін көрсетті. Патогеннің өсу аймағын шектеу диаметрі 9 мм-ден 48 мм құраған. Ең жоғарғы антагонисттік белсенділікті *Bacillus amyloliquefaciens* (48 мм) танытты.

**Түйін сөздер:** микробтық ценоз, *Erwinia amylovora*, жеміс дақылдарының бактериялық күйігі, антагонист-бактериялар, биобакылау.

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### **СТРУКТУРА МИКРОБНОГО ЦЕНОЗА ФИЛЛОСФЕРЫ ПОРАЖЕННЫХ БАКТЕРИАЛЬНЫМ ОЖОГОМ ПЛОДОВЫХ КУЛЬТУР В ЮЖНЫХ РЕГИОНАХ КАЗАХСТАНА**

**Аннотация.** Исследована структура и разнообразие микробных ценозов филлосферы яблони и груши в зонах промышленного садоводства Казахстана, где ранее наблюдалось поражение плодовых деревьев бактериальным ожогом. Исследования проводились в Жамбылской, Южно-Казахстанской и Алматинской областях. Из филлосферы здоровых и пораженных органов плодовых деревьев было отобрано 47 образцов (побеги, листья, плоды). В целом, выделено 216 микробных изолятов. На основе культурально-морфологических характеристик проведена таксономическая идентификация культур микроорганизмов. Изоляты были сгруппированы в следующие роды: бактерии (142 изолята): *Erwinia*, *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Paenibacillus*; грибы (37 изолятов): *Monilia*, *Venturium*, *Aspergillus*, *Podosphaera*, *Penicillium*, *Fusarium*, *Triothecium*, *Mucor*, *Verticillium*, *Alternaria*; дрожжи и дрожжеподобные микроорганизмы (37 изолятов): *Cryptococcus*, *Rhodotorula*, *Pullularia*, *Saccharomyces*. Бактерии доминировали в составе микробценозов. Во

всех исследованных образцах, отобранных из пораженных органов, присутствовали бактерии родов *Erwinia* и *Pseudomonas*.

Проведенный скрининг на антагонистическую активность к *E. amylovora* показал, что отдельные виды родов *Bacillus*, *Pseudomonas* и *Paenibacillus* продемонстрировали ингибирующее действие. Диаметры зоны подавления роста патогена составляли от 9 до 48 мм. Максимальную антагонистическую активность проявил *Bacillus amyloliquefaciens* (48 мм).

**Ключевые слова:** микробный ценоз, *Erwinia amylovora*, бактериальный ожог плодовых культур, бактерии-антагонисты, биоконтроль.

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