The article presents data on the isolation of 7 forage and grain crops from agrocenoses (soy, barley, alfalfa, rapeseed, safflower, donut and esparcet) of bacteria and the study of their PGPB properties. PGPB properties were evaluated in isolated bacteria by studying the ability to dissolve phosphates, nitrogen-fixing activity, antagonistic activity against the phytopathogen Fusarium graminearum, the ability to synthesize IUC. Determination of bacteria and actinobacteria before the genus was carried out by studying morphological, physiological and biochemical properties.

Main results. It was found that 659 different bacterial isolates were isolated from agrocenoses of various agricultural plants, while a significant part of the isolated isolates had a complex of properties (up to 30% of the strains show a pronounced ability to produce IUC), which may provide biocontrol and growth-stimulating effects. Among the isolated isolates, 191 are classified as gram-negative, 216 – non-spore-forming gram-positive, 136 – actinobacteria, 117 – as gram-positive spore-forming bacteria of the genus Bacillus. During the work, 7 promising strains were selected, representatives of the genera Agromyces, Bacillus, Streptomyces, which have a complex of properties: growth-stimulating, phosphate-dissolving, nitrogen-fixing and antagonistic activities for further development of highly effective microbiological preparations for crop production on their basis.

Key words: soil bacteria, agrocenosis, phosphate-solubilizing activity, nitrogen-fixing activity, antagonistic activity, synthesis of IUK.

PLANT GROWTH-PROMOTING BACTERIA ISOLATED FROM AGROCENOPSES OF AGRICULTURAL PLANTS
Стимулирующие рост растения бактерии, выделенные из агроценозов сельскохозяйственных растений

В статье представлены данные о выделении из агроценозов 7 кормовых и зерновых культур (сои, ячменя, люцерны, рапса, сафлора, донника и эспарцета) бактерий и изучение у них PGPB свойств. PGPB свойства оценивали у выделенных бактерий, изучая способность к растворению фосфатов, азотфиксирующую активность, антагонистическую активность в отношении фитопатогена Fusarium graminearum, способность к синтезу ИУК. Определение бактерий и актинобактерий до рода проводили, изучая морфологические, физиологические и биохимические свойства. Основные результаты. Установлено, что из агроценозов различных сельскохозяйственных растений выделено 659 различных бактериальных изолятов, при этом значительная часть выделенных изолятов обладала комплексом свойств (до 30 % штаммов проявляют выраженную способность продуцировать ИУК), которые, возможно, обеспечивают биоконтрольные и ростстимулирующие эффекты. Среди выделенных изолятов, 191 отнесены к грамотрицательным, 216 – неспорообразующим грамположительным, 136 – актинобактериям, 117 – к грамположительным спорообразующим бактериям рода Bacillus. В ходе работы было отобрано 7 перспективных штаммов, представителей родов Agromyces, Bacillus, Streptomycyes, обладающих комплексом свойств: ростстимулирующей, фосфатрастворяющей, азотфиксирующей и антагонистической активностями для дальнейшего создания на их основе высокоэффективных микробиологических препаратов для растениеводства.

Ключевые слова: почвенные бактерии, агроценоз, фосфатсолюбилизирующая активность, азотфиксирующая активность, антагонистическая активность, синтез ИУК.

Introduction

Developing sustainable and ecological methods to increase agricultural productivity without increasing the area of cultivated soil has become a critical issue for ensuring food safety. Among the various tools used to increase productivity in agriculture, the use of plant growth-promoting bacteria (PGPB) has great potential [1].

Plant growth-promoting bacteria (or PGPB) belong to a useful and heterogeneous group of microorganisms that can be found in soil, rhizosphere, root surfaces or plant tissues, they are able to enhance plant growth and protect them from disease as well as abiotic stress [2-5]. The mechanisms by which PGPB stimulates plant growth include nutrient availability, biological nitrogen fixation and phosphate solubilization, stress relief by modulating ACC deaminase expression and phytohormone and siderophore production, and others.

The relations between plants and microorganisms has been well studied for a long time, but their use in agriculture for partial or complete replacement of chemical fertilizers is still insufficiently studied [6]. The use of microorganisms as plant inoculants is one of the most important sustainable practices in agriculture. The positive effect of inoculation depends on a number of factors: on the activity of microorganisms; on the quantity and quality of synthesized substances by microorganisms; indigenous soil microflora; the inoculation method. It has been established that the best introduction is carried out by microorganisms originally isolated from plant agroecosynthesis. Nevertheless, despite the large number of studies devoted to the use of PGPB as useful inoculants for agricultural plants, most researchers note that the use of PGPB is poorly understood. Some researchers note the lack of effect of inoculum application, they associate this with insufficient study of the structure, composition and function of microorganisms of plant agroecosynthesis, therefore, it is necessary to study their ecology, physiology and biochemistry to assess the role of PGPB and the suitability of their use [7, 8].

The aim of this study is to isolate bacteria from plant agroecosynthesis that may have potential as environmentally friendly biofertilizers. The main directions of research: selection of strains with the ability to dissolve insoluble phosphates, to have nitrogen-fixation and antagonistic activities, and to produce IAA among isolated bacteria.

Materials and methods

The objects of research are microorganisms of different taxonomic groups isolated from plant agroecosynthesis.
Standard culture media and methods described in the manual [9] were used for the isolation of bacteria and actinobacteria from plant agrocnoses.

Methods for determining PGPB properties

Phosphate dissolving ability of microorganisms was studied by the formation of zones of dissolution of calcium phosphates around the colonies [10]. The ability to phosphate mobilization was assessed by calculating the IS solubilization index using the formula (IS = clearing zone diameter / colony diameter) and by the efficiency of ES solubilization according to the formula (ES = clearing zone diameter / colony diameter × 100).

Nitrogen fixation ability to of isolates was carried out by growing in a nitrogen-free medium [9].

The study of antagonistic activity was carried out by the method of double cultures [11]. Plates with phytopathogenic Fusarium graminearum without inoculation of the test isolates were used as a control. Determining the distance from the point of phytopathogen inoculation to the edge of the isolate colony.

To study the isolated strains properties that promote plant growth, we determined the calorimetric method using the Salkowski reagent in a liquid PDA medium with the addition of 2 mM L-tryptophan [12, 13].

Determination of bacteria and actinobacteria to genus was carried out according to the traditional scheme based on morphological, physiological, and biochemical properties [14].

Results and their discussion

The taxonomic composition of agrocnosis bacteria depends on the type of soil, temperature, humidity, light, and other factors. At the same time, in addition to these main factors that determine both the functional and spatial distribution of bacteria, the generic and species composition of bacterial communities depends on the degree of floristic saturation of biocenoses, their spatial position, the amplitude of vertical and horizontal distribution, the nature of individual species of life forms, and the biochemical structure of dominant species. Each type of soil and plant association is characterized by a specific composition of the bacterial community [15, 16, 17].

In general, bacterial diversity in agrocnoses is represented mainly by species belonging to Proteobacteria, Firmicutes and Actinobacteria phylae, where the most common genera known to include Bacillus, Pseudomonas, Enterobacter, Arthrobacter, Rhizobium, Agrobacterium, Burkholderia, Azospirillum, Mycobacterium, Flavobacterium, Cellulomonas and Micrococcus. Various researchers note that soil microbial communities of agrocnoses are dynamic, capable of significant changes in time due to seasonality, they are influenced by local environmental conditions and form recognizable types of structure of microbial communities [18-21].

In the studies carried out, a number of similar features and general patterns in the distribution of the bacterial community in soil samples from the studied agrocnoses were revealed. At the same time, the confinement of soils to a certain type of vegetation and the content of organic matter caused certain differences in the bacterial community. So, while studying the microbial community of soils under: barley, clover, green beans, soybeans, millet, the researchers noted that the soils under barley had the highest content of actinomycetes and fluorescent pseudomonads. The highest microbial activity was characteristic of the soils under barley and corn. The fungi / bacteria ratio was highest in soils under barley and lowest in soils under soybeans and potatoes. These data demonstrate that different cultures have different effects on microbial communities [22].

Soil suspensions of various dilutions were plated on solid media in order to isolate microorganisms, plates on which more than 30 colonies grew, were used for isolation. Morphologically different colonies were chosen and used for further research. 659 isolates were isolated from plant agrocnoses. The number of colony morphotypes was insignificant and amounted to no more than 15. Table 1 shows the quantitative ratio of isolates depending on the plant species and taxonomic belonging of the isolated isolates. Among the isolated isolates, 191 were classified as gram-negative, 216 – non-spore-forming gram-positive, 136 – actinobacteria, 117 – as gram-positive spore-forming bacteria of the genus Bacillus. The largest number of isolates was isolated from barley agrocnosis – 81 out of 659, and the smallest number of isolates was selected from rapeseed agrocnosis. The obtained data shows that more gram-positive non-spore-forming bacteria were isolated from plant agrocnoses than actinomycetes and spore-forming bacteria. The groups of isolated microorganisms can be arranged in the following order: gram-positive non-spore-forming bacteria > gram-negative bacteria > actinobacteria > spore-forming bacteria.
The use of microbial inoculants with phosphate-solubilizing activity in soils is considered as an environmentally friendly alternative to the use of chemical phosphorus fertilizers.

One of the reasons limiting the productivity of agricultural plants in Kazakhstan is the deficiency of two main nutrients in soils – nitrogen and phosphorus. Plants can use small amounts of phosphates from chemical sources, because 75-90% of the added phosphorus is precipitated through metal-cationic complexes and quickly becomes fixed in soils [23]. It is believed that inoculation of phosphate-solubilizing microorganisms into the soil is an effective way of converting insoluble phosphorus compounds into a form accessible to plants, leading to better plant growth and yield. Representatives of various genera, such as, for example, *Bacillus, Pseudomonas, Rhizobium, Aspergillus, Penicillium* are the most effective phosphorus solubilizers to increase its bioavailability in soil. Phosphate-solubilizing microorganisms not only promote plant growth by providing an easily assimilable form of phosphorus, they are able to synthesize plant growth hormones such as IAA, support plant growth through the production of siderophores and increase the efficiency of nitrogen fixation, and can also act as a biocontrol against plant pathogens [24-26]. And therefore, at the first stage of the study, we selected isolates with phosphate-solubilizing activity.

Screening of microorganisms for phosphate-solubilizing activity in 659 isolates made it possible to select 354 isolates with the ability to mobilize hardly soluble phosphates *in vitro* (Table 2).

**Table 1** – Component composition of the bacterial community from plant agrocenoses

| Plants               | Number of isolates | Total | Gram- bacteria | Gram + bacteria | actino-bacteria | spore-forming bacteria |
|----------------------|--------------------|-------|----------------|-----------------|-----------------|-----------------------|
| Barley               |                    | 81    | 23             | 28              | 16              | 14                    |
| Lucern of the first year |                  | 76    | 23             | 21              | 18              | 13                    |
| Lucern of the second year |                | 78    | 22             | 29              | 15              | 12                    |
| Lucern of the third year |                 | 74    | 18             | 29              | 15              | 12                    |
| Sainfoin             |                    | 74    | 19             | 28              | 14              | 13                    |
| Soya bean            |                    | 72    | 24             | 21              | 16              | 13                    |
| Safflower            |                    | 71    | 22             | 20              | 15              | 14                    |
| Clover               |                    | 68    | 21             | 20              | 14              | 13                    |
| Colza                |                    | 65    | 19             | 20              | 13              | 13                    |
| Total                |                    | 659   | 191            | 216             | 136             | 117                   |

**Table 2** – Component composition of the bacterial community with phosphate-solubilizing activity from plant agrocoenosis

| Plants               | Number of isolates | Total | Gram- bacteria | Gram + bacteria | Actino-bacteria | Spore-forming bacteria |
|----------------------|--------------------|-------|----------------|-----------------|-----------------|-----------------------|
| Barley               |                    | 50    | 13             | 15              | 10              | 12                    |
| Lucern of the first year |                  | 41    | 10             | 14              | 8               | 9                     |
| Lucern of the second year |                | 37    | 9              | 13              | 7               | 8                     |
| Lucern of the third year |                 | 47    | 11             | 16              | 11              | 8                     |
| Sainfoin             |                    | 37    | 11             | 11              | 6               | 9                     |
| Soya bean            |                    | 34    | 8              | 11              | 7               | 8                     |
| Safflower            |                    | 36    | 9              | 10              | 8               | 9                     |
| Clover               |                    | 36    | 11             | 9               | 7               | 9                     |
| Colza                |                    | 36    | 9              | 10              | 8               | 9                     |
| Total                |                    | 354   | 91             | 109             | 72              | 82                    |
The largest number of isolates with phosphate-solubilizing activity was isolated from the agrocoenosis of third year lucern and barley. The smallest number of isolates with the ability to mobilize phosphorus can be noted for soya beans. Among the selected isolates, the largest number belongs to selected gram-positive non-spore-forming bacteria.

In further studies, nitrogen-fixation activity was studied in 354 selected isolates. It was shown that 130 isolates had this activity, and the largest number of isolates was noted for third year lucern (Figure 1). Among the isolates, the largest number with nitrogen-fixation activity was selected in spore-forming bacteria and gram-negative bacteria.

The antagonistic properties of microorganisms with phosphate-solubilizing and nitrogen-fixing activities are presumably associated with competitive conditions in nutrient-poor ecological niches [27, 28]. Therefore, in our opinion, such microorganisms should be a potential target for the search of a new active antagonist strain.

Screening for antagonistic activity was performed by the block method. 45 were selected of the 130 studied, which gave clearly visible zones of no growth of the test culture. The highest activity was observed in 12 isolates, with the maximum zones of suppression of the growth of phytopathogens (from 16.2 to 25.2 mm). 3 isolates are spore-forming bacteria, 2 are gram-positive non-spore-forming bacteria, and 7 are actinobacteria.

Indole-3-acetic acid (IAA) is one of the widespread phytohormones in nature, the most active in the group of auxins. Auxins affect the division and differentiation of plant cells and tissues, stimulate the germination of seeds and tubers, promote xylem and root formation, are responsible for tropism, flowering and fruiting of plants, affect photosynthesis, pigment formation, biosynthesis of various metabolites, and plant resistance to stress factors [29, 30].

The detection and quantitative assessment of phytohormones of the auxin group was carried out by the colorimetric method using the Salkovsky reagent. Thus, out of 23 studied bacteria, 7 isolates showed a positive reaction to IAA production. IAA was most intensively produced by 4 isolates from 11.2 ± 1.4 μg / ml, but one isolate, in which the total production level reached 28.7 ± 2.1 μg / ml, is a particularly active producer of auxins. Ability to produce IAA among 17 strains of actinobacteria, 10 active isolates were identified, producing IAA in an amount from 11.0 μg / ml to 115 μg / ml (Table 3). The most active isolates were isolated from agrocoenosis of third year lucern and barley.

Screening of isolates with a complex of valuable properties made it possible to select a number of promising bacterial strains isolated from plant agrocoenosis. Initial identification revealed a wide variety of isolated microorganisms. The dominant groups of bacteria with PGPB properties among the studied microorganisms were representatives of the genera Bacillus and Streptomyces (Table 4).
The largest number of active bacteria was isolated from the agrocoenosis of two plants, barley and the third year lucern. The obtained results in this research indicate a high potential for the search for microorganisms with PGPB characteristics of plant agrocoenosis. The selection of promising microorganisms is a complex process, in this study, the screening was carried out taking into account the productivity of plants and therefore paid attention to such properties as growth-stimulating, phosphate-dissolving, nitrogen-fixation and antagonistic activities.

**Conclusion**

It was found that 659 different bacterial isolates were isolated from agrocoenosis of various agricultural plants, while a significant part of the
isolated isolates had a set of properties (more than 40% of bacteria exhibit pronounced properties and a number of strains are capable of actively producing IAA), which, possibly, provide biocontrol and growth-stimulating effects. During the research, 7 promising strains were selected, representatives of the genera Agromyces, Bacillus Streptomyces, possessing a set of properties: growth-stimulating, phosphate-dissolving, nitrogen-fixation and antagonistic activities for further creation on their basis of highly effective microbiological preparations for plant growing.

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Conflict of interest

The authors have no conflicts of interest.

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