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Өсімдіктердің биологиясы және биотехнологиясы институтының

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## ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК  
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## NEWS

OF THE NATIONAL ACADEMY OF SCIENCES  
OF THE REPUBLIC OF KAZAKHSTAN  
of the Institute of Plant Biology and Biotechnology

**БИОЛОГИЯ ЖӘНЕ МЕДИЦИНА  
СЕРИЯСЫ**



**СЕРИЯ**

**БИОЛОГИЧЕСКАЯ И МЕДИЦИНСКАЯ**



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**RESEARCHES ON THE DEVELOPMENT OF BIOADDITIVES BASED ON CONSORTIA OF SOIL MICROORGANISMS**

**Abstract.** A new promising direction of increasing the yield and quality of agricultural products, preserving and replenishing the natural fertility of soils is the development of biologically active additives with organic and organomineral fertilizers.

The purpose of our research was to develop and optimize the technology of obtaining directional bioadditives, study the patterns and effectiveness of their effects on plants. Bioadditives based on consortia of soil microorganisms providing for accelerated growth of plants, improving their qualitative characteristics, as well as increasing the fertility of poor soils were developed and tested.

The technology of fermentation of aerobic cultivation of the following bioadditives based on consortia of soil microorganisms *Azotobacter vinelandii*, *Beijerinckia fluminensis*, *Bacillus cereus* and *Bacillus macerans* has been worked out. In our researches, we used a less complex mineral composition, which ensures a good growth of cultures and accumulation of the necessary amount of active biomass of bacteria. Cultivation of consortia was carried out in a laboratory fermenter. Sowing of cultures in a nutrient medium was carried out simultaneously. During cultivation samples of a cultural liquid were selected and the quantity of each kind of bacteria of the consortium was determined in them. The determination was carried out by dilution and seeding on a solid nutrient medium (Koch method). A consortium of nitrogen-fixing bacteria was plated on a nitrogen-free medium (Ashby), a consortium of a mineral of dissolving bacteria on  $MPA + CA = 1:1$ .

The results of the experiment showed that the concentration of cells in the resulting preparation was  $10^{13}$  cells/ml. Of these, 25% was attributed to *Azotobacter vinelandii*, 30% to *Beijerinckia fluminensis*, 20% to *Bacillus macerans*, and 25% to *Bacillus cereus*.

**Keywords:** biofertilizers, bacterial biomass, mold fungi, a consortium of microorganisms, a liquid nutrient medium, mineral fertilizers, *Azotobacter vinelandii*, *Beijerinckia fluminensis*, *Bacillus cereus*, *Bacillus macerans*.

**Introduction.** As the development of agricultural ecosystems created to maximize production, the impact on nature caused by the redistribution of energy and matter on the surface of the Earth is constantly increasing. The perfection of labor tools, the introduction of high-yielding crops and varieties that require a large number of nutrients, began to violate the natural processes [1-5].

Unreasonable methods of protecting crops and the system of land are devastating, soil erosion and loss of fertility due to irrational use and non-compliance with preventive measures and technology of soil protection, contamination of surface and groundwater with residues of pesticides and nitrates coming from agricultural lands; and much more [6-10].

To regulate and solve these problems, scientifically based techniques and methods are needed that, in certain cases, can only partially prevent or reduce the undesirable effects that arise in the production of primary biological products under various management conditions [11-15].

One of the most promising approaches to this task is the development of bioadditives based on consortia of soil microorganisms and plant growth regulators for the intensification of plant growing. Since ancient times, people have used soil microorganisms to increase crop yields. Prior to the appearance of chemically bound nitrogen in the cultivation of bread or the use of pastures, people could rely on

replenishing the soil with nitrogen only as a result of the activity of microorganisms. Based on soil bacteria in the XX century, such fertilizers as azotobacterin and phosphorobacterin were created [16-19].

**The aim** of the research work is to study and develop biotechnology of bioadditives based on a consortium of soil microorganisms and plant growth regulators

### Research methods

**Study the growth kinetics of bacterial cultures.** To determine the growth parameters of the microorganisms bioadditives and the optimal fermentation time of *Azotobacter vinelandii*, *Beijerinckia fluminensis*, *Bacillus cereus* and *Bacillus macerans*, the kinetics of their cultivation was studied in laboratory experiments.

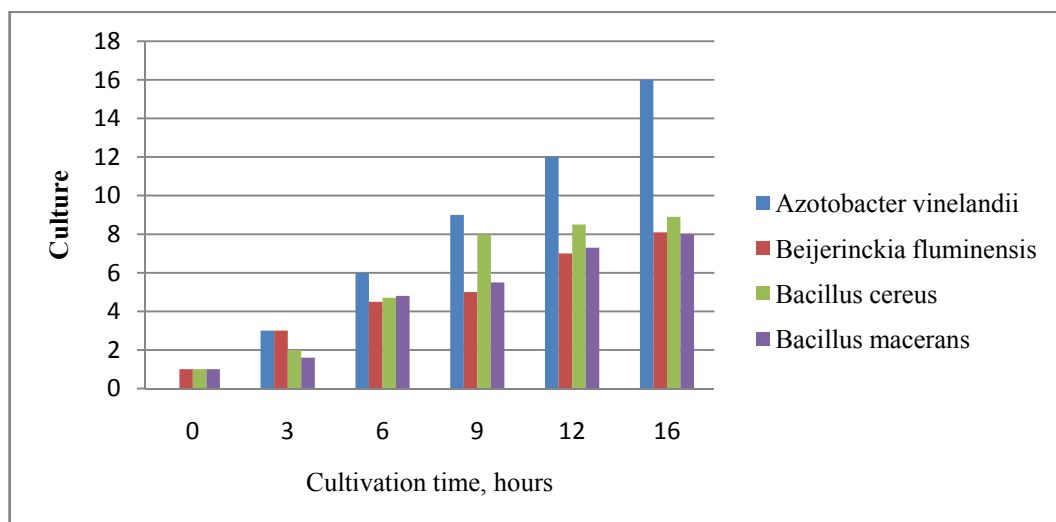
In the study of the growth dynamics of *Azotobacter vinelandii*, *Beijerinckia fluminensis*, *Bacillus cereus*, and *Bacillus macerans*, a series of shaker experiments and a laboratory unit with a volume of 1 liter were performed with aeration and medium stirring at a mass exchange level of 4.0 kg O<sub>2</sub>/m<sup>3</sup>h. The composition of the mineral medium was used the same as for analyzing the effect of the carbonaceous substrate on the cultivation process. As the carbon source for all four cultures, sucrose was used at a concentration of 3 g/l.

### Results and discussion

When sampling, microscopy of samples with drug staining was performed, which showed the presence of at least 90% of living cells in them. All growth curves of bacterial cultures, obtained from experimental data (table 1, figure), have a typical S-shape.

Table 1 – Dynamics of biomass accumulation (g/l) of bacteria when cultivated on a liquid nutrient medium

Culture	Cultivation time, hours							
	0	3	6	9	12	16	20	25
<i>Azotobacter vinelandii</i>	0	3	6	9	12	16	20	25
<i>Beijerinckia fluminensis</i>	1	3	4,5	5	7	8,1	8,3	8,4
<i>Bacillus cereus</i>	1	2	4,7	8	8,5	8,9	9,5	9,8
<i>Bacillus macerans</i>	1	1,6	4,8	5,5	7,3	8	8,8	10



Bacterial biomass accumulation in cultivation in a liquid medium

However, due to the complexity of the composition and the absence of such a component as the juice of soybean seedlings, which is used in significant quantities (10%), it is not advisable to recommend this compound for industrial production in our region.

In our studies, we used a less complex mineral composition, which ensures a good growth of cultures and accumulation of the necessary amount of active biomass of bacteria.

Cultivation of consortia was carried out in a laboratory fermenter. Sowing of cultures in a nutrient medium was carried out simultaneously. During cultivation samples of a cultural liquid were selected and the quantity of each kind of bacteria of the consortium was determined in them. The determination was carried out by dilution and seeding on a solid nutrient medium (Koch method). A consortium of nitrogen-fixing bacteria was plated on a nitrogen-free medium (Ashby), a consortium of a mineral of dissolving bacteria on MPA + CA = 1: 1. The repetition of the experiment is threefold. The averaged results are presented in tables 2 and 3.

Table 2 – Dynamics of nitrogen-fixing bacteria in cultivation in a consortium on a liquid nutrient medium (Cl/ml)

Option	Cultivation time, hours							
	0	3	3	9	12	16	20	25
Consortium: <i>Beijerinckia fluminensis</i> <i>Azotobacter vinelandii</i>	1,2·10 <sup>3</sup>	2·10 <sup>5</sup>	10·10 <sup>5</sup>	7·10 <sup>6</sup>	6,5·10 <sup>8</sup>	2·10 <sup>8</sup>	7·10 <sup>11</sup>	7·10 <sup>13</sup>
	6·10 <sup>4</sup>	1·10 <sup>5</sup>	5·10 <sup>5</sup>	4·10 <sup>6</sup>	4·10 <sup>8</sup>	1·10 <sup>11</sup>	4·10 <sup>11</sup>	4·10 <sup>13</sup>
	8·10 <sup>4</sup>	1·10 <sup>5</sup>	4·10 <sup>5</sup>	2·10 <sup>6</sup>	2·10 <sup>8</sup>	10·10 <sup>10</sup>	3·10 <sup>11</sup>	3·10 <sup>13</sup>

Table 3 – Dynamics of the numbers of *Bacillus cereus* and *Bacillus macerans* in a consortium on a liquid nutrient medium (Cl/mL)

Option	Cultivation time, hours							
	Cultivation time, hours	3	3	9	12	16	20	25
Consortium: <i>Bacillus cereus</i>	0	3	5	7	13	21	24	30
	1·10 <sup>5</sup>	2·10 <sup>5</sup>	2·10 <sup>6</sup>	7·10 <sup>6</sup>	3·10 <sup>8</sup>	8·10 <sup>10</sup>	2·10 <sup>11</sup>	2·10 <sup>13</sup>
	7·10 <sup>4</sup>	1·10 <sup>5</sup>	4·10 <sup>5</sup>	1·10 <sup>7</sup>	2·10 <sup>6</sup>	4·10 <sup>10</sup>	1·10 <sup>11</sup>	1·10 <sup>13</sup>
<i>Bacillus macerans</i>	9·10 <sup>3</sup>	1·10 <sup>5</sup>	5·10 <sup>8</sup>	2·10 <sup>6</sup>	1·10 <sup>7</sup>	3·10 <sup>10</sup>	1·10 <sup>11</sup>	1·10 <sup>13</sup>

Previously, for the joint cultivation of *Azotobacter vinelandii* and *Bacillus mucilaginosus*, a nutrient medium composition was proposed that includes %: soybean-10 seed juice, 0.5 sucrose, 0.5 starch, yeast extract -0.005, MgSO<sub>4</sub> -0.02, K<sub>2</sub>HPO<sub>4</sub> -0.1, NaCl-0.02, CaCO<sub>3</sub> -0.3, a solution of microelements-0.3, vitamin-B1-3 ppm, vitamin B12-3 ppm.

The solution of microelements includes,%: Na<sub>2</sub>MoO<sub>4</sub>-0.5, H<sub>3</sub>BO<sub>3</sub>-0.5, FeCl<sub>3</sub> -0.5.

In our studies, we used a less complex mineral composition, which ensures a good growth of cultures and the accumulation of the necessary amount of active biomass of bacteria. Cultivation of consortia was carried out in a laboratory fermenter. Sowing of cultures in a nutrient medium was carried out simultaneously. During cultivation samples of a cultural liquid were selected and the quantity of each kind of bacteria of the consortium was determined in them. The determination was carried out by dilution and seeding on a solid nutrient medium (Koch method). A consortium of nitrogen-fixing bacteria was plated on a nitrogen-free medium(Ashby), a consortium of mineral dissolving bacteria on MPA + CA = 1: 1 (figure 1).

The repetition of the experiment is threefold. The averaged results, dynamics of nitrogen-fixing bacteria in cultivation in a consortium on a liquid nutrient medium are presented in tables 2 and 3.

**Conclusion.** The experiment was carried out without growing the plants. Before applying the bioadditives, the soil was moistened to a humidity of 70-75% and kept at room temperature (20-22 °C) for 24 hours. Bioadditive for the experiment was developed in the laboratory. The concentration of cells in the resulting preparation was 10<sup>13</sup> cells/ml. Of these, 25 % were *Azotobacter vinelandii*, 30% - *Beijerinckia fluminensis*, 20% - *Bacillus macerans*, 25 % - *Bacillus cereus*.

The seventh day in the control conditions, the number of fungi and bacteria was practically the same and amounted to 1·10 cells/g of soil, respectively, with one bacterium dominated by one species of rod-shaped bacteria with dilapidated colonies.

The introduction of bioadditives into the soil caused a decrease in the fungal microflorasoil (0.4·10 cells/g) and an increase in the number of bacteria (20·10 cells/g), and the species diversity of bacteria also increased.

The introduction of compost in the soil led to an increase in the number of microflora and, to a greater extent, mushroom. The number of fungi and bacteria in

This variant was 4·10 and 6·10 cells/g.

The joint application of bioadditives and compost to the soil promoted the increase in the number of bacteria in comparison with the control, compost and bioadditive. The number of bacteria in variants No. 4 and No. 5 was 64·10 cells/g and 84 cells/g, respectively, of fungi -2·10 and 3·10 cells/g, respectively.

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**ТОПЫРАҚ МИКРОАҒЗАЛАР КОНСОРЦИУМЫНА НЕГІЗДЕЛГЕН  
БИОҚОСПАЛАР ЖАСАУ НЕГІЗІН ЗЕРТТЕУ**

**Аннотация.** Биологиялық белсенді қоспаларды және органикалық және органикалық минералды тыңайтқыштарды дамыту ауылшаруашылық өнімдерінің сапасын арттыру, топырақтың табиғи құнарлылығын сақтау және толтыру үшін жаңа перспективалық бағыт болып табылады.

Зерттеудің мақсаты - әсері бар биокосымшаларды алу технологиясын әзірлеу және оңтайландыру, өсімдіктерге олардың тиімді әсерін және заңдылықтарын зерттеу болып келеді. Биокосымшалар өсімдіктердің тез өсуін қамтамасыз ететін топырақ микроорганизмдерінің консорциумы негізінде әзірленді және сыналды, олардың сапалық сипаттамаларын жақсартты, сондай-ақ нашар топырақтардың құнарлылығын жоғарылатты.

Топырақ микроорганизмдерінің *Azotobacter vinelandii*, *Beijerinckia fluminensis*, *Bacillus cereus* и *Bacillus pasteurii* консорциумдарына негізделген келесі биобиокосымшаларды аэробты өсіру ашыту технологиясы әзірленді. Жүргізілген зерттеуге сәйкес, минералды қоспаның күрделі қоспасын қолдану ұсынылды, ал бұл бактерия биомассасының жақсы өсіп-дамуына көп мөлшерде жинақталуына мүмкіндік береді. Консорциумды өсіру лабораториялық ферменттерде жүргізілді. Қоректік ортаға культураны егу бір мезгілде өтті. Өсіру барысында үлгі дақылдық сұйықтықтан алынып, бактериялар консорциумының әрбір жекелеген түріне олардың мөлшеріне зерттеулер жүргізілді. Анықтау барысы сұйылту әдісіне сай, қатты ортаға қайта егу арқылы жүзеге асырылады (Кох әдісі). Азот фиксирлеуші бактериялардың консорциумын азоты жоқ Эшби ортасына егу МПА-СА = 1:1 минералды ыдырату бактериялары консорциумына сай жүргізілді.



Корытынды тәжірибелер көрсеткендей, алынған бактерия жасушаларының концентрациясы  $10^{13}$  кл/мл артты. Оның ішінде 25% *Azotobacter vinelandii*, 30% - *Beijerinckia fluminensis*, 20% - *Bacillus macerans*, 25% - *Bacillus cereus* тұрады.

**Түйін сөздер:** биотыңайтқыштар, бактерия биомассасы, саңырауқұлақтар, микроағза консорциумы, сұйық коректік орта, минералды тыңайтқыштар, *Azotobacter vinelandii*, *Beijerinckia fluminensis*, *Bacillus cereus*, *Bacillus macerans*.

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### ИЗУЧЕНИЯ РАЗРАБОТКИ БИОДОБАВКИ НА ОСНОВЕ КОНСОРЦИУМОВ ПОЧВЕННЫХ МИКРООРГАНИЗМОВ

**Аннотация.** Новым перспективным направлением повышения урожайности и качество сельскохозяйственной продукции, сохранения и восполнения естественного плодородия почв является разработка биологически активных добавок и органическим и органо- минеральным удобрениям.

Целью наших исследований была разработка и оптимизация технологии получения биодобавок направленного действия, изучение закономерностей и эффективности воздействия на растения. Были разработаны и испытаны биодобавки на основе консорциумов почвенных микроорганизмов обеспечивающие ускоренный рост растений, улучшения их качественных характеристик, а также повышающие плодородия бедных почв.

Отработано технология ферментации аэробного культивирования следующих биодобавок на основе консорциумов почвенных микроорганизмов *Azotobacter vinelandii*, *Beijerinckia fluminensis*, *Bacillus cereus* *Bacillus macerans*. Согласно исследованию, было предложено использовать сложную смесь минеральных удобрений, которая позволяла бактерии биомассы накапливаться в больших количествах. Разведение консорциума проводили в лабораторных ферментах. Одновременно проводили посев культуры в питательных средах. Во время культивирования образец извлекали из культивируемой жидкости и исследовали для каждого отдельного вида бактериальных консорциумов и их размера. Процесс обнаружения проводили путем рециркуляции в твердую среду (метод Кох) в соответствии с методом разбавления. Концентрат бактерий азота, закрепляющий азот, сделан посев в среде Эшби без азота в соответствии с бактериальным консорциумом для разложения минерального разложения МРА-Са 1: 1.

Результаты эксперимента показали, что концентрация клеток в полученном препарате составляла  $10^{13}$  кл/мл. Из них 25% приходилось на *Azotobacter vinelandii*, 30% - *Beijerinckia fluminensis*, 20% - *Bacillus macerans*, 25% - *Bacillus cereus*.

**Ключевые слова:** биоудобрения, бактериальная биомасса, плесневые грибы, консорциум микроорганизмов, жидкая питательная среда, минеральные удобрения, *Azotobacter vinelandii*, *Beijerinckia fluminensis*, *Bacillus cereus*, *Bacillus macerans*.

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