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DEVELOPMENT OF EFFECTIVE STRAINS OF NODULE BACTERIA AND MICROBIAL PREPARATIONS BASED ON THEM

Introduction. *Preparations based on highly effective and competitive strains of nodule bacteria improve nitrogen and phosphorus nutrition of plants, are a source of biologically active compounds. They are environment friendly, have a high selective effect and aftereffect, increase the yield and enhance the stress-resistance of legumes.*

Problem Statement. *With the intensification of chemicalization of agriculture, the level of environment pollution is increasing while the quality of food is deteriorating. In this regard, it is important to search new, scientifically sound approaches to developing modern management systems that will ensure the production of environment friendly plant products. The most advantageous way to solve this problem today is the optimization of plant-microbial interactions, one of the types of which is legume-rhizobial symbiosis.*

Purpose. *The purpose of this research is obtaining highly effective strains of rhizobia with the use of the cutting-edge molecular biology and nanobiotechnologies and developing innovative microbial preparations on their basis for inoculation of legume seeds.*

Materials and Methods. *Strains of nodule bacteria of alfalfa, goatweed, soybean, pea and lupine and S17-1 Escherichia coli strain with different plasmid vectors have been used. Methods of analytical selection, microbiological, physiological, and statistical have been applied.*

Results. *Highly effective competitive strains of rhizobia for basic legumes have been obtained. They provide a yield increase of 11–21% as compared with the control strains. New generation preparations Rhizostym and Rhizostym-M, which are complex inoculants of binary action on the basis of nodule bacteria and additional biological agents, have been developed.*

Conclusions. *The developed microbiological innovative preparations provide a significant economic effect. They are used for obtaining environmentally friendly products, preserving and reclaiming soil fertility. These properties determine the prospects for their use in agricultural production.*

Keywords: nodule bacteria, inoculation, legume-rhizobial symbiosis, strain, bacterial preparations, Rhizostym.

Given a significant dependence of agriculture in Ukraine on climatic and environment conditions, as well as its determinism by socio-economic factors, environment safety in this industry is becoming a key issue in the Ukrainian society. Therefore, research aiming

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at finding a new, scientifically sound approach to the creation of modern management systems that would ensure the production of environment friendly plant products is very relevant. In this regard, industrialized countries, despite affordability of mineral fertilizers, pay special attention towards the biologization of agricultural production. The most expedient way to solve the outlined problem today is to intensify the formation of plant-microbial interaction, in particular, legume-rhizobial symbiosis as one of its types. Therefore, a promising task of modern agriculture is to maximize the use and to increase the symbiotic potential of legumes, in particular, through the development of highly effective biological products of microbial origin, including bacterial fertilizers that not only improve mineral nitrogen and phosphorus nutrition, but also are a source of biologically active compounds (vitamins, hormones, enzymes, antibiotics, etc.). Bacterial preparations based on microorganisms do not pollute the environment, are safe, show high selective action and aftereffect. They are complex fertilizers, as the microorganisms that are part of them not only fix atmospheric nitrogen or transform sparingly soluble phosphates, but also synthesize compounds with growth-regulating and phytoprotective action [1—3].

Pre-sowing inoculation of legume seeds is a mandatory biotechnological method that promotes the formation of high yields. Inoculation of seeds or introduction of beneficial microorganisms into the soil through the use of specially developed microbiological preparations based on created highly effective competitive strains of nodule bacteria helps to improve the growth and development of legumes, increases their yield due to stimulating seed germination, improving plant nutrition, triggering photosynthetic activity, increasing their resistance to pathogens and abiotic stresses [3].

Insofar as monoinoculants are more sensitive to adverse environment factors, stabilization and optimization of agronomically beneficial effects of bacterial preparations by inoculation of legume seeds is achieved through the complex action of biological agents (components of preparations such as bacteria with different environment functions, natural substances, biological chelated forms of plant nutrients, metal carboxylates, etc.). Thus, the realization of the symbiotic potential of nodule bacteria can be enhanced by creating complex microbial bio-preparations based on microorganisms with different agronomically useful properties: nitrogen fixation, ability to transform sparingly soluble phosphorus compounds, bioremediation of soils, synthesis of growth-regulating agents and phyto-protectors. The use of complex microbiological preparations that provide more stable yields as compared with the monoinoculants, in particular under the action of stress factors of different nature, is an effective and promising biotechnological element in the legume growing technology [2, 4].

At the same time, the molecular genetic studies of biological nitrogen fixation and the active development of modern technologies have allowed finding new ways to improve the effectiveness of biological preparations of nitrogen-fixing microorganisms with the use of:

- Graphite-talc media. This technology provides excellent seed flow in the grain sowing and protects the seeds from spoiling [5];
- Composition of strains of nitrogen-fixing microorganisms with contrast properties. This approach provides a high complementarity of components of the preparations to plant varieties of different maturity groups and the effectiveness of fertilizers under the action of adverse environment factors [3, 6, 7];
- Special bioadhesives. The technology is based on using, together with the inoculant, polymer compounds that provide additional protection of rhizobia cells from pesticides, moisture deficiency, and excessive temperature. This allows preliminary treatment of legume seeds 2—3 months before sowing [8—10];
- Lipochitooligosaccharide (LCO) promoters. This technology is based on accelerating the formation of symbioses through the use of LCO

signaling. Lipochitooligosaccharide signaling molecules activate a cascade of biochemical reactions that aim at activating the formation of symbiotic systems and overcoming varietal incompatibility of micro- and macro-partners [11—14];

- Plant proteins with lectin activity and bacterial polysaccharides. The use of these signaling molecules activates the cascade of biochemical reactions involved in the formation and functioning of symbiotic systems [3, 15—19].

The purpose of this research is to obtain new highly effective strains of rhizobia through using advanced molecular biology and nanobiotechnologies, as well as to develop on their basis modern innovative microbial biological products for inoculation of legume seeds.

The experiments used strains of nodule bacteria of alfalfa, goatweed, soybean, pea, and lupine and strain S17-1 *Escherichia coli* with different plasmid vectors pSUP2021 :: Tn5 (*Simon R., Priefer U., Puhler A.*, 1983) [20] or pSUP5011 :: Tn5*mob* (*Simon R., O'Connell M., Labes M., Puhler A.*, 1986) [21] from the collection of nitrogen-fixing microorganisms of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine (IPPG of the NAS of Ukraine), which has the status of national asset. Plasmids have antibiotic resistance factors, including kanamycin (Km) at a concentration of 200 μg/ml encoded by Tn5 transposon.

The Tn5 transposon consists of two almost identical inverted sequences IS50L and IS50R. IS50R encodes the transposase and its inhibitor. The vector construction contains the Km antibiotic resistance gene.

The selection of parent cells from wild strains (counterselection) allows the resulting conjugates to be removed from the parent cells because the parent cells cannot grow on media with a high concentration of antibiotic. *E. coli* is counterselected on TY medium with the addition of 800—1000 μg/ml streptomycin (Str), while the wild strains of rhizobia is counterselected on YMA medium with the addition of 200 μg/ml kanamycin. Rhizobia strains involved in Tn5 mutagenesis shall have a low resistance to Km, in the range of 25—50 μg/ml.

Nodule bacteria are grown for three days at a temperature of 28 °C on agar medium 79: 0.5 g/l distilled water; $K_2HPO_4 \times H_2O$; 0.2 g/l $MgSO_4 \times 7H_2O$; 0.1 g/l NaCl; traces of $CaCO_3$; 2 g/l yeast extract; 0.5 g/l casamic acid or lactalbumin; 10 g/l mannitol; pH of the medium is 7.2—7.4; sterilization at 0.8—1 atm for 30 min. *E. coli* S17-1 is cultured for one day at 37 °C on LB medium [22].

Competent recipient strains shall be sensitive to 25—50 μg/ml Km on culture medium and resistant to 800—1000 μg/ml Str. The Tn5 transposon is introduced into rhizobia cells with the use of *E. coli* S17-1 according to the method described in [23], as modified by the authors. Bacterial cultures of donor and recipient are mixed at a ratio of 1:5 in 0.5 ml sterile water. The mix of cells is transferred to agar medium TY [22] in Petri dishes and incubated at +28 °C for 6—30 hours. The bacteria are washed from the surface of the medium with sterile water (5 ml) and suspended. Sequential 10-, 100-fold, etc. dilutions of the conjugate mix of cells are prepared and seeded on selective medium of 0.2 ml. TY + 200 Km + 500 Str (μg/ml) used as a selective medium for the selection of Tn5 mutants. Cell counterselection (selection against *E. coli* donor strain) is done on TY + 800 μg/ml Str medium.

The transposition frequency (V) is calculated by the ratio:

$$V = \frac{\text{Number of cells grown on TY + Km + Str in the 0-dilution}}{\text{Number of cells grown on TY + Str in } x\text{-th dilution}}$$

Because of conjugation with *E. coli*, rhizobia become able to reproduce on selective YMA medium with kanamycin (200 μg/ml).

One of the methods for obtaining economically valuable strains of nodule bacteria is analytical selection that to date has remained practically significant [24—25]. The source material is rhizobia extracted from root nodules of plants or directly from microbial soil coenoses.

Effective competitive strains of nodule bacteria are selected in pot and field conditions.

The pot experiments are carried out on a specially equipped site of IPPG of the NAS of Ukraine, the field experiments are done in different soil and climatic conditions of Kyiv, Cherkasy, Ternopil, and Lviv Oblasts.

To study the stability of combined biological products based on rhizobia and trace elements under the conditions of their long-term storage, the liquid bacterial preparation *Rhizostym* has been modified. Thus, Ge and Fe nanocarboxylates are added to the YMA medium at a concentration of 1 : 1 : 1000.

Original *Rhizostym* preparation (without any additional substances) is taken as control one. Having been sterilized, the medium is kept for a week and checked for spontaneous contamination.

The number of microorganisms (in terms of optical density) is determined with the use of standard methods and a spectrophotometer *BIORAD SmartSpecPlus* (USA) at a wavelength of 600 nm. Optical density is measured on the third and fourth days of cultivation.

The titer in the finished bacterial fertilizers is measured on the day of manufacture of the preparation and after its long-term storage (30 and 45 days).

Further, the effect of long-term storage of the modified *Rhizostym* (*Rhizostym*-M) has been studied in Kyiv Oblast at the research site of the Research and Production Department *Alexandria* of the Institute for Plant Protection of the NAAS of Ukraine and in the Cherkasy Oblast on the basis of agrobiostation of Pavlo Tychyna Uman State Pedagogical University. *Almaz* soybean variety seeds are treated with: 1) freshly prepared original preparation, 2) freshly prepared modified preparation, and 3) modified preparation after long-term storage. Before sowing into the soil, the seeds are inoculated with a suspension of the above rhizobial cultures having the same titer (10^8 cells in 1 ml inoculum). Soybean seeds are sown at a rate of 600 thousand similar seeds per 1 ha, to a depth of 3—5 cm, the width between rows is 45 cm, and the plot area is 15 m^2. All experiments are done in ac-

Table 1. **New Highly Effective Competitive Strains of Nitrogen-Fixing Microorganisms**

Strain	Selection method	Green mass		Grain		Note
		Additive to producing strain				
		cwt/ha	%	cwt/ha	%	
Rhizobium leguminosarum bv. viciae M1	Transposon mutagenesis (*E. coli* pSUP2021::Tn5)	—	—	3.6—5.2	11—15	Patent for invention № 81577
Sinorhizobium meliloti T17 (B—7282)	Transposon mutagenesis (*E. coli* pSUP2021::Tn5)	17—48	10—15	—	—	Utility model patent № 55432
Bradyrhizobium japonicum T21-2 (B—7322)	Transposon mutagenesis (*E. coli* pSUP2021::Tn5)			3.5—6.4	13—20	Patent for invention № 64086
Bradyrhizobium japonicum PC08 (B—7399)	Analytical selection	—	—	3.7—6.5	14—21	Utility model patent № 78775
Bradyrhizobium japonicum B-20 (B—7538)	Transposon mutagenesis (*E. coli* pSUP5011::Tn5*mob*)	—	—	4.2—4.6	15—19	Utility model patent № 126060
Sinorhizobium meliloti AC08 (B—7411)	Analytical selection	43—59	11—13	—	—	Patent for invention № 111391
Rhizobium galegae K50	Transposon mutagenesis (*E. coli* pSUP5011::Tn5*mob*)	16—67	13—20	—	—	Utility model patent № 138542
Rhizobium leguminosarum bv. *viciae* 7П	Analytical selection	—	—	3.0—4.3	11—18	Prepared for patenting
Bradyrhizobium sp. (*Lupinus*) 168-5	Transposon mutagenesis (*E. coli* pSUP2021::Tn5)			2.1—3.2	14—21	Prepared for patenting

cordance with the generally accepted agricultural techniques. Sampling for research is carried out during budding and flowering.

Statistical processing of experimental data has been done according to the method by B. A. Dospekhov [26], with the use of *Microsoft Excel* 2017.

Over the last 10 years, more than 20 strains of nitrogen-fixing microorganisms have been created with the use of analytical selection and genetic engineering methods by researchers of the IPPG of the NAS of Ukraine. The developments have been protected by copyright certificates and patents of Ukraine for inventions (Table 1).

The obtained rhizobia have unique contrasting symbiotic properties. Among them, there are strains with a high nitrogen-fixing activity, which contribute to the formation of quality crops with a high protein content, and are characterized by a high complementarity (have a much greater degree of interaction) to plant varieties of Ukrainian or foreign selection, regionalized in Ukraine, which significantly increases the effectiveness of bacterial preparations in soil and climatic conditions of our country. Especially valuable are the strains of microorganisms, which through the application of nanobiotechnological approaches have acquired new economically valuable features, the effectiveness and stability of which have been proven by multi-year tests. They are able to survive and to form an effective symbiosis with the host plant in stressful conditions, which is especially important for modern agricultural production.

As a result of multi-year research, highly effective competitive strains of nodule bacteria have been selected for the main legumes. They provide a yield increase of 11—21% as compared with control strains. Among them, strains that are characterized by increased resistance to adverse environment conditions (low positive temperature, lack of moisture, excessive nitrogen in the soil, and pesticides) play a special part.

The use of pre-sowing seed treatment with inoculants on the basis of pesticide-resistant active strains of rhizobia *B. japonicum* PC07, PC10, and B20 in the form of monoinoculation and complex (multistrain) preparations of different composition provides an increase in the crop yield of *Almaz* soybean variety by 4.9—6.0 kg/ha (Table 2).

Thus, the functioning of legume-rhizobial symbioses formed with active, fungicide-resistant strains of rhizobia *B. japonicum* PC07, PC10, and B20, with different methods of their application (mono-, binary, multistrain inoculation) and a high level of providing plants with bound atmospheric nitrogen in the phase of active assimilation processes has a positive effect on plant growth and development and on grain productivity of soybean during the growing season.

For the last 20 years, the Institute of Plant Physiology and Genetics of the NAS of Ukraine has been actively working to create and to study the effectiveness of complex inoculants for grain legume crops and perennial legumes. The creation of such compositions is based on the principle of individual selection and maximum complementarity of components. They may contain the following components: culture of soil nitrogen-fixing microorganisms (nodule bacteria or rhizosphere diazotrophs) that provide the plant with environment friendly biological nitrogen and are a source of biologically active substances (hormones, vitamins, amino acids, etc.) and fungistatic and antibacterial substances; biologically active substances of plant or bacterial origin, which have a wide

Table 2. **Yield of Soybean in the Case Inoculation with Pesticide-Resistant Strains of Nodule Bacteria (field experiment)**

Treatment	Yield, cwt/ha		
	Total yield	Increase in the experiment	
		cwt/ha	%
Control *B. japonicum* 6346	22.6 ± 1.3	—	—
B. japonicum PC07	28.2 ± 1.4	+ 5.6	+ 24.8
B. japonicum PC10	28.6 ± 1.4	+ 6.0	+ 26.5
B. japonicum PC07 + + PC10	27.5 ± 1.2	+ 4.9	+ 21.7
B. japonicum PC07 + + PC10 + B20	28.0 ± 1.2	+ 5.4	+ 23.9

range of biological activity, have a positive effect on the development of bacterial culture in the composition thereby increasing the inoculation load of the final preparation and ensuring maximum potential of plant-bacterial systems by activating important symbiotic properties of microorganisms. Methods of enhancing the symbiotic properties of microorganisms underlying the creation of complex compositions are protected by Patents of Ukraine [15, 27].

With the use of classical and modern technologies, *Rhizostym* and *Rhizostym-M* preparations of new generation (liquid and powder forms) for legumes have been created. The biological basis of the preparations is patented highly effective strains of soybean and pea nodule bacteria that are characterized by increased production of exopolysaccharides and resistance to adverse environment factors, have a higher nitrogen-fixing activity (1.5—2.0 times) and a higher virulence (by 10—20%), contribute to an increase in crop yield by 12.0—14.5% as compared with control preparations. After harvesting, a high content of nitrogen compounds of organic origin remains in the soil and in crop residues. The high effectiveness of *Rhizostym* and *Rhizostym-M* is ensured by a complex of biopolymer compounds of plant origin, which includes soybean or pea seed lectin that accelerates the formation of symbiosis, activates the process of biological fixation of molecular nitrogen and increases plant resistance to adverse environment factors. This improves the adhesion of bacteria to the seeds and prolongs their storage on the surface of the seeds. The microbial preparations based on the strains of nodule bacteria resistant to modern fungicides allow the early seed treatment and the use of inoculum in combination with plant protection agents in seed treatment [3, 28].

Several substances have been used as additional agents in the development of compositions based on nodule bacteria.

1. PGPR-bacteria of the genera *Azotobacter* and *Enterobacter,* which are obtained by the method for analytical selection from chernozem soil and rhizosphere zone of spring and winter wheat. Representatives of these taxons are characterized by a set of positive effects on the system plant — soil — microorganisms, in particular on the ability to fix molecular nitrogen, breakage of sparingly soluble phosphates, xenobiotics, synthesis of substances with growth-stimulating and phytoprotective action. It has been established that the seed productivity of soybean variety *Mariana* increases, on average, by 17% in the field conditions, and by 13% and 14% in pot conditions for varieties *Mariana* and *Annushka*, respectively, as compared with the monoinoculation of seeds by rhizobia with inoculation of seeds with binary bacterial composition soybean rhizobia + azotobacter.

It has been proved that the use of inoculum based on soybean rhizobia and bacteria of the *Enterobacter* genus on the roots of soybeans there are formed nodules that are 2.2—2.9 times larger in terms of weight and 1.5—3.5 times higher in terms of functional activity as compared with the control samples in the case of rhizobia monoinoculation. The plants bring by 20% more pods. This indicates an increased realization of their productive potential [4, 29, 30].

2. Extracts of blue-green algae *Nostoc commune* and *Nostoc punctiforme*, which are capable of producing biologically active metabolites with growth-activating and antioxidant effects. Creating joint algorhizobial associations that include culture of blue-green algae and effective strains of nodule bacteria may be a way to biologically stimulate legume-rhizobial symbiosis. The use of *N. commune* for growing alfalfa enhances interaction of rhizobia with plants and the effectiveness of bacterial preparations based on them by 5—15% (Fig. 1).

It should be noted that the complex bacterization of legume seeds by these microorganisms may be effective only in the case of selection of compatible inoculation partners. In addition to useful biologically active substances (phytohormones, vitamins, polysaccharides), blue-green algae emit antibiotics and toxic substances, which are likely to adversely affect the bacterial cells of certain strains of rhizobia [31—33].

3. Compounds of carbohydrate nature, in particular, glucose-containing monosaccharides (glucose and N-acetyl-D-glucosamine) that may be synthesized by plant and excreted with root exudates in the rhizosphere and affect the development and physiological activity of microsymbionts. It has been found that the use of a complex preparation based on soybean nodule bacteria and glucose-containing monosaccharides (glucose, N-acetyl-D-glucosamine) for inoculation of soybean seeds of early-maturing varieties *Lisabon* and *Almaz* results in a significant increase in the degree of rhizobia nodulation capacity realization, as the number of nodules grows 1.6—2.2 and 1.4—2.3 times, respectively, as compared with the action of monoinoculant; the weight of nodules increases 1.4—2.3 and 1.9—2.1 times, respectively; and the nitrogen-fixing activity rises 1.7—2.1 times, respectively. The yield of soybean seeds significantly increases by 12—14% as compared with the inoculation only with rhizobia. The seed productivity grows due to a significant increase in such indices of crop structure as the number of pods per plant (by 13 and 15%) and the weight of seeds per plant (by 20%). These results have outlined the prospects for the use of glucose and acetylated amino sugars as exogenous regulatory agents for the formation, functioning, and effectiveness of soybean-rhizobial symbioses and may be used for modifying the rhizobial inoculum for soybean with the help of these compounds of carbohydrate nature [3, 30].

4. Carboxylates of nanoparticles of metals (germanium, iron and molybdenum) as components of inoculation suspension, which provide not only the active formation and functioning of the symbiotic mechanism, but also the preservation of physiological activity of bacteria during long-term storage, which allows making the preparation in advance . It is known that metal carboxylates, due to the small particle size, are characterized by a high specific surface area and, consequently, by a high reactivity [34]. The nanoparticles have been proved to affect biological objects at the cellular level through being involved in the processes of electron transfer and to raise the effectiveness of physiological processes in plants [35, 36].

The results have shown that after long-term storage of preparations there is reported an increase in the titer of rhizobia in the environment of their cultivation (Table 3), which depends on

Table 3. **Effect of Long-Term Storage on the Rhizobia Titer in the Preparations**

Variant	Rhizobia titer, CFU × 10^9		
	on the day of preparation	in 30 days	in 45 days
Original preparation	4.5 ± 0.1	5.2 ± 0.3	4.2 ± 0.4
Modified preparation (Ge + Fe)	4.7 ± 0.2	5.7 ± 0.1	5.0 ± 0.1

Note: CFU is colony forming units.

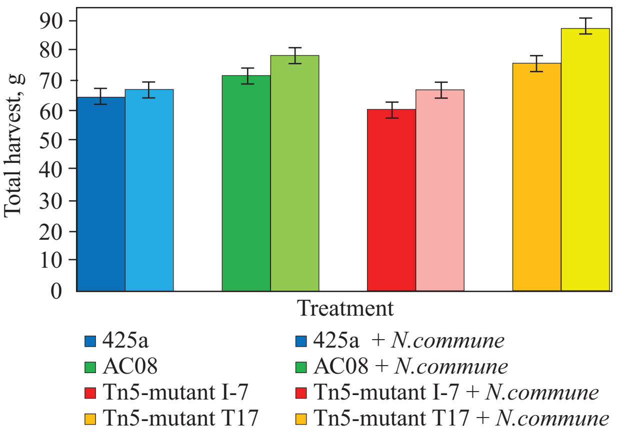

Fig. 1. Productivity of green mass of *Yaroslavna* variety of alfalfa inoculated with mixed suspensions on the basis of blue-green algae and nodule bacteria (pot experiment)

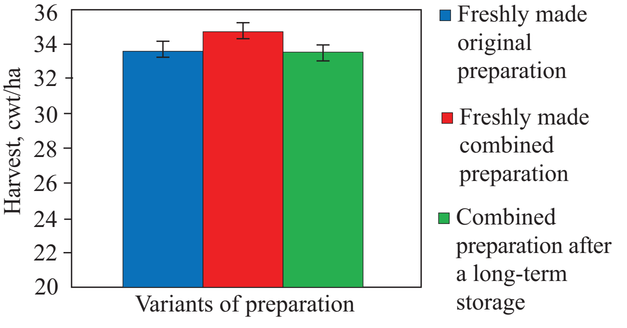

Fig. 2. Yield of *Almaz* soybean grown in the field conditions in the case of treatment with combined bacterial preparations

the shelf life and the type of preparation. Thus, on the 30th day of storage, there is a trend towards increasing the titer of rhizobial cells in both original and modified preparations. The modified preparation is characterized by a more intense growth in the cell biomass as compared with the reference one. The further storage of the preparation for up to 45 days leads to a slight decrease in the titer of rhizobia in both studies. In the modified preparation, this effect is less remarkable, and the number of microorganisms is kept at the level of rhizobia titer on the day of its preparation.

The next stage of research is to study the effect of treatment with modified preparation *Rhizostym* (freshly made and after a long-term storage) on the formation of symbiotic structures (weight and number of nodules) and their functioning (nitrogen-fixing activity) in field experiment. The effect of three variants of the preparation has been studied: variant 1 — freshly made original preparation; variant 2 — freshly made combined preparation; variant 3 — combined preparation after a long-term storage (Table 4).

Based on the obtained results, the highest number, mass of nodules, and their nitrogen-fixing activity in the budding stage are reported in the case of treatment with freshly prepared combined preparation. Inoculation of soybean seeds with freshly prepared (original) preparation and combined preparation after long-term storage has the same effect on the nodulation process and the intensity of nitrogen fixation. The same trend is maintained during the flowering stage of plants, but the studied parameters in plants treated with the modified preparation after long-term storage insignificantly decrease (within 6%) as compared with the case of treatment with the original preparation.

Table 4. **Formation and Functioning of Symbiotic Apparatus of Soybean Variety *Almaz* Inoculated with Combined Bacterial Preparations and Grown in Field Conditions**

Variant	Number of nodules, units/plant	Weight of nodules, g/plant	NFA, nmol C_2H_4/(plant × h)
	Budding Stage		
1	4.60 ± 1.34	0.12 ± 0.01	1.52 ± 0.29
2	9.11 ± 1.21	0.21 ± 0.01	2.63 ± 0.21
3	6.52 ± 1.48	0.16 ± 0.01	1.57 ± 0.18
	Flowering Stage		
1	9.80 ± 1.89	0.49 ± 0.07	2.05 ± 0.23
2	10.25 ± 1.64	0.56 ± 0.04	2.44 ± 0.21
3	9.63 ± 2.09	0.42 ± 0.06	1.92 ± 0.16

Note: NFA is nitrogen-fixing activity.

The analysis of the productivity of soybean plants has shown that the value of this parameter does not depend on the duration of storage of the preparation (Fig. 2). The analysis of the harvest has displayed that the effectiveness of different forms of the preparation in pre-sowing treatment is almost the same. The yield is slightly higher with the use of freshly made combined preparation.

Thus, as a result of the research, the preparation based on modern highly effective competitive strains of *B. japonicum* and nanocarboxylates of germanium and iron has been developed. Despite long-term storage (30 and 45 days), it remains effective for pre-sowing inoculation of soybean seeds and ensures the formation of a powerful symbiotic mechanism in experimental plants.

Modification of the preparation with germanium and ferrum nanocarboxylates not only guarantees the active formation and functioning of the symbiotic mechanism in plants, but also ensures the preservation of physiological activity of bacteria during their long-term storage, which allows making the preparation in advance without reducing its effectiveness.

Conclusions

With the use of methods of analytical selection and genetic engineering, highly effective competitive, resistant to adverse environmental factors strains of nodule bacteria for the main legumes have been obtained. They provide a yield increase of 11—21% as compared with the control strains.

Innovative microbial preparations of new generation *Rhizostym* and *Rhizostym-M* (liquid and powder forms) for legumes have been developed with the use of classical and modern technologies. The biological basis of these preparations is new active and highly effective strains of rhizobia.

Complex binary inoculants based on nodule bacteria and additional biological agents (rhizobacteria, plant and microbial metabolites, algae, biologically active substances of protein and carbohydrate nature, carboxylates of metal nanoparticles) are promising biotechnological elements to be used not only for increasing plant yields, but also for obtaining environment friendly products, preserving and reclaiming soil fertility. Such microbiological innovative preparations of complex action provide a significant economic effect and contribute to the improvement of phytosanitary condition of soils by reducing the chemical load on the ecosystem, which determines the prospects for their use in agricultural production.

The study has been conducted as part of targeted research program of the Department of General Biology of the NAS of Ukraine *Fundamentals of Forecasting and Preventing Negative Effects of Climate Change on Biotic Systems of Ukraine* (KPKVK 6541030), project *The Development of Biotechnological Products for Regulating the Adaptability of Soybean and Rhizobia into Production* and target complex program for fundamental research of the NAS of Ukraine *Fundamental Problems of the Creation of New Nanomaterials and Nanotechnologies* (KPKVK 6541030), project *The Development of a Complex of Nanocarboxylates of Microelements with Microbial Preparations and Methods for Its Application in the Newest Cultivation Agrotechnologies.*

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СТВОРЕННЯ ЕФЕКТИВНИХ ШТАМІВ БУЛЬБОЧКОВИХ БАКТЕРІЙ ТА МІКРОБНИХ ПРЕПАРАТІВ НА ЇХ ОСНОВІ

Вступ. Препарати на основі високоефективних і конкурентоспроможних штамів бульбочкових бактерій поліпшують азотне й фосфорне живлення рослин, слугують джерелом біологічно активних сполук, є екологічно безпечними, проявляють високу селективну дію та післядію, підвищують урожайність та стресостійкість бобових культур.

Проблематика. Із посиленням хімізації сільськогосподарського виробництва зростає рівень забруднення довкілля та погіршується якість продуктів харчування. Тому актуальним є пошук нових, науково обґрунтованих підходів до створення сучасних систем господарювання, які забезпечать виробництво екологічно чистої рослинної продукції. Доцільним шляхом вирішення проблеми на сьогодні є оптимізація рослинно-мікробних взаємодій, одним із видів яких є бобово-ризобіальний симбіоз.

Мета. Отримання високоефективних штамів ризобій сучасними засобами молекулярної біології та нанобіотехнології та розробка на їхній основі інноваційних мікробних препаратів для інокуляції насіння бобових культур.

Матеріали й методи. Використано штами бульбочкових бактерій люцерни, козлятника, сої, гороху і люпину та штам S17-1 *Escherichia coli* з різними плазмідними векторами. Застосовано методи аналітичної селекції, мікробіологічні, фізіологічні та статистичні.

Результати. Отримано високоефективні конкурентоспроможні штами ризобій під основні бобові культури, які забезпечують зростання урожаю на 11—21% порівняно зі штамами-стандартами. Розроблено препарати нового покоління «Ризостим» та «Ризостим-М», які є комплексними інокулянтами бінарної дії на основі бульбочкових бактерій та додаткових біоагентів.

Висновки. Створені мікробіологічні інноваційні препарати забезпечують істотний економічний ефект, спрямовані на отримання екологічно чистої продукції, збереження й відтворення родючості ґрунтів, що зумовлює перспективність їхнього використання у сільськогосподарському виробництві.

Ключові слова: бульбочкові бактерії, інокуляція, бобово-ризобіальний симбіоз, штам, бактеріальні препарати, «Ризостим».