JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

FREE-FLOWING COMPLEX BACTERIAL PREPARATION FOR CROP AND EFFICIENCY OF ITS USE IN AGROECOSYSTEMS

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doi: 10.15414/jmbfs.2015.4.6.527-531

ARTICLE INFO

Received 14. 1. 2015
Revised 2. 4. 2015
Accepted 6. 4. 2015
Published 1. 6. 2015

Regular article

OPEN ACCESS

ABSTRACT

The present study investigated the influence of different nanomaterials on physiological and biochemical activity of the nitrogen-fixing bacteria *Azotobacter vinelandii* IMV V-7076 and phosphate mobilizing bacteria *Bacillus subtilis* IMV V-7023 for the development of high-efficient free-flowing bacterial complex for crop production. Among the studied nanomaterials, vermiculite stimulated the most effectively bacterial growth, synthesis of amino acids and phytohormones, dehydrogenase, catalase and peroxidase activities. Based on vermiculite and highly efficient strains of bacteria *Azotobacter vinelandii* IMV V-7076 and *Bacillus subtilis* IMV V-7023, a free-flowing bacterial complex preparation for crop production was created. The preparation was stable during storage, it improved the nitrogenous and phosphorus nutrition of plants stimulated their growth by biologically active substances and protected plants from lesion by phytopathogenic micromicetes and bacteria.

Keywords: Complex bacterial preparation, nanoparticles, *Azotobacter vinelandii*, *Bacillus subtilis*, antioxidant enzymes, phytohormones

INTRODUCTION

Soil microorganisms are one of the main factors of its fertility. At the same time they can cause adverse effects on plant growth and development and cause their diseases. Therefore, an important goal of plant-growing is correction of microbial processes in agroecosystems through the use of effective bacterial preparations (**Kurdish, 2010**). This will reduce the use of chemicals in agriculture and have high quality of plant products.

The majority of proposed in the last decade of microbial preparations is made on the basis of individual monoculture in the form of suspensions. The number of viable microorganisms in them quickly reduced (**Kameneva *et al.*, 2008**), which negatively affects the quality of preparations for their storage. It is known**,** that the complex microbial preparations made on the basis of two or more strains of microorganisms have more noticeable influence on growth, development and crop yield of plants (**Kurdish, 2001; Titova *et al.*, 2001; Kasem, 2004**).

The high-efficiency strains of nitrogen-fixing bacteria *Azotobacter vinelandii* IMV V-7076 (**Patent № 72856**) and phosphate mobilizing bacteria *Bacillus subtilis* IMV V-7023 (**Patent № 54923A**) selected in our previous studies. Based on their interaction with the clay mineral bentonite, granulated complex bacterial preparation for crop production Azogran was created. It has a stable composition during prolonged storage, improves nitrogenous and phosphorous nutrition of plants, stimulates their growth and development by the biologically active substances of bacterial origin, protects plants against pathogens and increases their yield crop on 18-37% (**Kurdish, 2010**). However the granules of preparation are badly suspended. Therefore for bacterization of seed cereals it would be better to use free-flowing preparation is based on these strains of bacteria and nanomaterials.

The aim of this study was to determine the regularities of influence of nanomaterials of the different nature on the functioning of the nitrogen-fixing bacteria *Azotobacter vinelandii* IMV V-7076 and phosphate mobilizing bacteria *Bacillus subtilis* IMV V-7023 for the creation of high-efficient free-flowing complex bacterial preparation for crop production.

MATERIAL AND METHODS

Microorganisms, nutrient media and culture conditions

The nitrogen-fixing bacteria *Azotobacter vinelandii* IMV V-7076 (**Patent № 72856**) and phosphate mobilizing bacteria *Bacillus subtilis* IMV V-7023 (**Patent № 54923A**) were isolated at the Department of Microbiological processes on solid surfaces, Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine. The strain *A. vinelandii* IMV V-7076 was grown in 750 mL Erlenmeyer flasks with 100 mL of Ashby's liquid medium (g/L): sucrose 20.0, $K_2HPO_4 \cdot 3H_2O$ 0.2, $MgSO_4 \cdot 7H_2O$ 0.2, NaCl 0.2, K_2SO_4 0.1, $CaCO_3$ 5.0, distilled water 1 L. This medium was supplemented with 1 mL solution of Fedorov microelements the following composition (g/L): H_3BO_3 5.0, $(NH_4)_2MoO_4 \cdot 2H_2O$ 5.0, $ZnSO_4 \cdot 7H_2O$ 0.2, KI – 0.5, NaBr 0.54, $Al_2(SO_4)_3 \cdot 18H_2O$ 0.3, distilled water 1 L (pH 7.2-7.3) (**Rubenchik, 1960**). The strain *A. vinelandii* IMV V-7076 was grown in liquid Berk's medium (g/L): $K_2HPO_4 \cdot 3H_2O$ 0.8, KH_2PO_4 0.2, sodium citrate 0.5, $CaCl_2$ 0.1, $MgSO_4$ $7H_2O$ 0.2, $FeSO_4$ $7H_2O$ 0.015, $Fe_2(SO_4)_3$ $9H_2O$ 0.005, Na_2MoO_4 $2H_2O$ 0.005, sucrose 20.0, distilled water 1 L (pH 7.0-7.4) (**Rubenchik, 1960**). The strain *B. subtilis* IMV V-7023 was grown in 750 mL Erlenmeyer flasks with 100 mL of glucose-mineral liquid medium (g/L): $(NH_4)_2SO_4$ 0.5, $MgSO_4$ $7H_2O$ 0.3, NaCl 0.3, KCl 0.3, $CaCO_3$ 5.0, $MnSO_4$ $7H_2O$ 0.001, $FeSO_4$ $7H_2O$ 0.001, glucose 10.0, inorganic phosphate salts (KH_2PO_4 and K_2HPO_4 $3H_2O$ in the ratio 1:1) 1,0 (0,6 PO_4^{3-}) (pH 7.0-7.4) (**Egorov, 1983**). Another liquid nutrient medium for *B. subtilis* IMV V-7023 consisted **of** (g/L): $(NH_4)_2SO_4$ 0.5, $MgSO_4 \cdot 7H_2O$ 0.3, KCl 0.3, $CaCO_3$ 5.0, $MnSO_4 \cdot 7H_2O$ 0.001, $FeSO_4$ 0.001, glucose 10.0, calcium glycerophosphate 2.0 (pH 6.8-7.2) (**Tepper, 1979**).

Pre-weighed of the nanoparticles were added before sterilization in the nutrient media. After sterilization the nutrient media were inoculated with respective strains. The initial bacterial concentration after inoculation was 10^5-10^6 cells/mL. Incubation was performed under batch conditions at 28 °C with shaking at 240 rpm for 24-48 h. The optimization of nutrient media for culturing bacteria was carried in the multifactor experiment (**Biryukov *et al.*, 1985**).

Cultural liquids were freed from the bacteria as described in the works (**Chobotarjov *et al.*, 2010; Herasimenko *et al.*, 2013; Skorochod *et al.*, 2013**).

The amino acids, phytohormones and enzyme activities were determined in the resulting cultural media.

Nanoparticles

In this study we used different nanoparticles: (1) silica nanoparticles with size of 5 – 20 nm (**Chuiko *et al.*, 1992**); (2) montmorillonite nanoparticles with a Dashukivski occurrence of bentonite clays with a size of 50 nm; (3) saponite nanoparticles with a Tashkivski occurrence of Khmelnitsky Region; (4) glauconite nanoparticles with a Maciorski occurrence of Khmelnitsky Region; (5) titanium dioxide nanoparticles; (6) vermiculite nanoparticles with size of 50 nm (**Tarasevich *et al.*, 1975**).

Determination of quantity of viable bacteria in free-flowing preparation

10 g of preparation was dissolved in 90 mL of physiological solution (pH 7.0). Incubation was performed with shaking at 240 rpm for 60 min. With the resulting suspension was done the 10-fold dilutions and plated in Petri dishes with the appropriate dilutions on Ashby's agar medium (for *A. vinelandii* IMV V-7076) and Potato agar medium (for *B. subtilis* IMV V-7023). The plates having 30-300 CFUs (colony forming units) were used for calculating the CFU in the samples.

Determination of amino acids

Content the sum of amino acids in the cultural media of *A. vinelandii* IMV V-7076 and *B. subtilis* IMV V-7023 was determined with a Biotronik LC-2000 analyzer (Biotronik, Frankfurt/Main, FRG).

Enzyme activity assay

Catalase activity in the culture medium of *B. subtilis* IMV V-7023 was estimated accordingly to the method of **Korolyuk *et al.* (1988)** using UV-46 spectrophotometer (joint stock company "LOMO (Leningrad Optical-Mechanical Association)", Russia). This method is based on the ability of hydrogen peroxide to form a stable, colored complex with molybdenum salts with absorption maximum at 410 nm by measuring of the breakdown of hydrogen peroxide. Catalase activity was expressed as mmol H_2O_2/min per mg protein.
Peroxidase activity in the culture medium of *B. subtilis* IMV V-7023 was measured accordingly to the method described by **Popov *et al* (1971)**. The method is based on photometric registration of decrease in the concentration indigo carmine, which is oxidized by hydrogen peroxide in the presence of peroxidase. The rate of indigo carmine oxidation was estimated spectrophotometrically at 610 nm. Peroxidase activity was expressed as mmol indigo carmine/min per mg protein.
Dehydrogenase activity in the culture medium of *B. subtilis* IMV V-7023 was measured accordingly to the method described by **Friedel *et al* (1994)**. The assay is based on the reduction of 1% 2.3.5-triphenyltetrazolium chloride (TTC) to red-colored triphenyl formazan (TF). Experiments were carried out under anaerobic conditions in Thunberg tubes. Dehydrogenase activity was expressed as mg TF/mg protein.
Protein concentration was estimated by the **Bradford** (**1976**) procedure using crystalline bovine albumin as standard.

Determination of phytohormones

The HPLC method with liquid chromatograph Agilent 1200 (Agilent Technologies, USA), equipped with the diode matrix detector, and analytical column Eclipse XDB-C 18 5 μm, 4,6x150 was used for analysis of phytohormones in the culture media of bacteria (**Methods of plant hormones identification. 1988. Kyiv: Institute of Botany AS of USSR, 78 p.)**

Effect of free-flowing complex preparation on growth and yield of cereals crops

The effectiveness of the impact of free-flowing granular complex bacterial preparation on the growth and yield of cereals crops was investigated for spring barley grade Nabat and winter wheat grade Tsarivna in collaboration with Institute of Feed Research and Agriculture of Podillya of the National Academy of Agrarian Sciences of Ukraine within three years. Experiments conducted on orthic grey wooded soil. Area was 25 m^2.

Statistical analysis

Microsoft excel (Microsoft corporation, USA) was used to analyze data on the average of three replicates (±SE) obtained from three independent experiments. Differences were compared for statistical significance at the P - level less than 0.05 ($P<0.05$). The Kolmogorov-Smirnov test was used to assess the normality of the distribution of each treatment **(Zar, 1984).**

RESULTS AND DISCUSSION

Effect of nanoparticles on the growth activity of *Bacillus subtilis* IMV V-7023 and *Azotobacter vinelandii* IMV V-7076

It was established that the interaction *B. subtilis* IMV V-7023 and *A. vinelandii* IMV V-7076 with nanoparticles of various natural and synthetic materials (saponite, vermiculite, glauconite, silica, titanium dioxide) stimulated physiological and biochemical activity of bacterial populations (**Chobotarjov *et al.*, 2010; Herasimenko *et al.*, 2013; Skorochod *et al.*, 2013**). In this study, we found that cultivation of these bacteria in the medium containing 1 g/L saponite led to 2-fold and 3-fold increase in the number of bacilli and *A. vinelandii* IMV V-7076 cells, respectively, compared to control ones (Figure 1). The most pronounced increase in CFUs was observed by cultivating with 10 g/L of saponite. Influence of saponite on the growth of microorganisms up to the present time was not explored. Therefore, it was assumed that saponite-induced increase in growth activity of bacteria can be due to the intensification of the substrate intake by microbial cells. However, the higher content of this mineral in the nutrient medium suppressed growth of these bacteria. It is known that the dispersed materials at certain concentrations can form on the surface of bacterial cells the layer of the adsorbed particles (**Globa *et al.*, 1983**). Perhaps, the presence of such a layer on the surface of bacteria creates a barrier which inhibits penetration of the substrate into cells that, in turn, can reduce the growth activity of microorganisms.

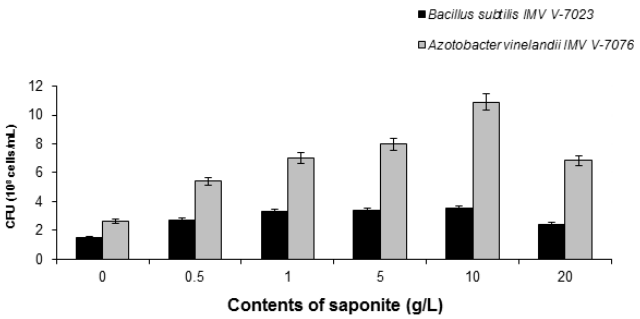

Figure 1 Effect of saponite on the growth activity of *Bacillus subtilis* IMV V-7023 and *Azotobacter vinelandii* IMV V-7076. Bacterial cultures were grown under batch conditions at 28 °C with shaking at 240 rpm for 48 h.

Significant stimulating effect on growth of studied microorganisms was rendered by the particles of exfoliated vermiculite (Table 1). Kameneva (2009) showed that vermiculite with particle size up to 1 mm (2 % of the volume of the medium) is an important technology component for the production of heterophase preparation of *Mesorhizobium ciceri* H-12 with bacterial titer of 6.9–8.0 billion/mL, maintenance of cell viability and nodular activity up to three months.
Stimulating effect of this mineral on the growth of *A. vinelandii* IMV V-7076 was defined in different culture media (Table 1). It was shown that at cultivation of *A. vinelandii* IMV V-7076 in liquid Berk's medium containing 5 – 10 g/L the particles of vermiculite, the amount of cells in increased by 18 – 20 %, in comparison with the control. In the Ashby's liquid medium, the population of *A. vinelandii* IMV V-7076 increased almost the order compared to control.
Adding of exfoliated vermiculite particles in the suspension of *B. subtilis* IMV V-7023 influenced also positively on the growth of these bacteria. Supplementation of medium with 5 g/L of vermiculite increased by 49 % the number of bacilli compared with the control cultures (Table 1). Dispersed particles of saponite and glauconite showed also stimulating effect on the growth of the used bacteria (**Chobotarjov *et al.*, 2010**).

Table 1 The number of *B. subtilis* IMV V-7023 and *A. vinelandii* ÌMV V-7076 **cells under** their cultivation in media with particles of vermiculite

Contents of vermiculite g/L	**The number of bacteria**		
	A. vinelandii ÌMV V-7076 CFU (10^8 cells/mL)		*B. subtilis* IMV V-7023 CFU (10^9 cells/mL)
	Nutrient media		
	liquid Berk's medium	Ashby's liquid medium	with calcium glycerophosphate and glucose
control	8.7±0.2	0.9±0.1	**0.6±0.3**
0.5	10.2±0.1	3.6±0.2	1.2±0.2
1.0	10.8±0.5	3.7±0.5	1.8±0.1
5.0	10.3±0.2	8.4±0.8	8.5±0.6
10.0	10.4±0.5	9.1±0.5	–
20.0	8.9±0.3	9.2±0.5	–

Notes: 1. Bacteria **were** cultivated **for** 48 hours; 2. the initial bacterial titer after inoculation was **1×10^6 cells/mL; 3. «–» – not determined**

Influence of nanomaterials on synthesis of some bioactive substances by bacteria

One of the most important properties of symbiotic, rhizospheric and epiphytic bacteria is an ability to stimulate and improve plant growth and development due to producing of phytohormones belonging to different classes: auxins, gibberellins, cytokinins, ethylene, and abscisic acid (Tsavkelova, 2006). The bacteria *B. subtilis* IMV V-7023 and *A. vinelandii* IMV V-7076 synthesize a number of biologically active substances (Tserkovniak, 2009). Synthesis of biologically active substances increased under cultivation of these bacteria with particles of nanomaterials (Table 2). It was found that accumulation of amino acids in the culture medium of *A. vinelandii* IMV V-7076 increased in 5 – 6 times in the nutrient medium containing 5 g/l of glauconite or saponite. However, synthesis of amino acids in *B. subtilis* IMV V-7023 was repressed by these minerals. At the same time, the content of amino acids in the culture medium of bacilli increased by 27.7 % by growth on titanium dioxide (Table 2).

Table 2 Effect of type of the dispersed material on accumulation of amino acids in the cultural media of *A. vinelandii* IMV V-7076 and *B. subtilis* IMV V-7023

Type of the dispersed material	**Total content of amino acids, µg/mL**	
	A. vinelandii IMV V-7076	*B. subtilis* IMV V-7023
Control	**2.76±0.13**	**11.80±0.51**
Titanium dioxide	**2.81±0.17**	**15.07±0.29**
Glauconite	**14.58±0.42**	**6.80±0.15**
Saponite	**16.67±0.38**	**3.46±0.19**

Notes: 1. Control was nutrient medium without dispersed material; 2. the content of dispersed materials in nutrient medium was 0.5 %

The significant increase in level of phytohormones was observed at cultivation of the bacteria with the different nanoparticles (Table 3). By the introducing of silica in nutrient medium the content of zeatin in culture medium of *B. subtilis* IMV V-7023 was increased by 85 % compared with the control. Visible stimulating effect on the synthesis of phytohormones by these bacteria was observed by using of vermiculite particles. It was found, that at cultivation of bacilli with 5 g/L of this mineral the content of zeatin in their culture medium was increased by 17 %, zeatin riboside - by ~ 20 % and zeatin glucoside - by 144 % (Table 3)

Table 3 The effect of nanomaterials on accumulation of cytokinins in the cultural medium of *B. subtilis* IMV V-7023

Phytohormones	**Content of phytohormones in the cultural medium of *Bacillus subtilis* IMV V-7023, ng/mL**		
	Control (without nanomaterials)	With silica	With vermiculite
Zeatin	**69.3±1.5**	**128.6±1.4**	**81.0±1.1**
Zeatin riboside	**94.6±1.7**	**102.0±1.1**	**113.1±1.7**
Zeatin glucoside	**89.1±1.5**	**35.0±1.7**	**217.2±1.9**

Effect of vermiculite nanoparticles on dehydrogenase activity and activity of antioxidant enzymes

The interaction **of** these strains of bacteria with vermiculite nanoparticles significantly increased their dehydrogenase activity, and also the activity of antioxidant enzymes. The introduction 0.5 – 10 g/L of this mineral in a nutrient medium of *A. vinelandii* IMV V-7076 increased of dehydrogenase activity more than by 40 % (Figure 2). However, the silica nanoparticles did not have a stimulating effect on the dehydrogenase activity of this strain. The similar effects of these nanomaterials on dehydrogenase activity of *B. subtilis* IMV V-7023 were observed (Figure 2).

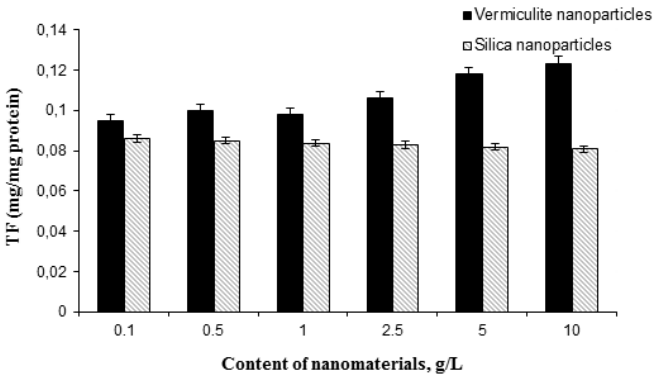

Figure 2 Dehydrogenase activity in cultural media of *Azotobacter vinelandii* IMV V-7076 cultivated with vermiculite nanoparticles and silica nanoparticles for 48 h. The culture medium of *A. vinelandii* IMV V-7076 without vermiculite nanoparticles was used as control. Dehydrogenase activity in control was 0.09 mg TF/mg protein.

The vermiculite nanoparticles influenced positively on the antioxidant enzyme activity of *B. subtilis* IMV V-7023. It was shown, that at addition of 1.5 and 2.5 g/L of this nanomaterial into a nutrient medium the peroxidase activity of bacteria increased to 3-fold. However, at higher content of vermiculite nanoparticles (5 g/L), the extracellular peroxidase activity was lower than at stimulating vermiculite levels (Table 4). The results are in a very good agreement with well-know principle in toxicology and medicine: „dose determines the mechanism". The toxicity of used compounds is connected with development with oxidation stress and depends not only on the level of the materials, but also on its surface and chemical composition (**Zhu Lin *et al.*, 2007**). Approximate formula of vermiculite is $(Mg^{+2}, Fe^{+2}, Fe^{+3})_3\ [(AlSi)_4O_{10}]\cdot(OH)_2\cdot4H_2O$. The chemical composition of vermiculite is 14 – 23 % MgO, 1 – 3 % FeO, 5 – 17 % Fe_2O_3, 10 – 13 % Al_2O_3, 37 – 42 % SiO_2, 8 – 18 % H_2O (**Kameneva, 2009**). Since the content of SiO_2 is sufficiently high, it can initiate the peroxide oxidation of biomolecules (**Gerashenko, 2009**), leading to activation of the extracellular peroxidase activity bacilli at the certain dose of vermiculite particles in a nutrient medium. However, with the increasing of content SiO_2 inhibits peroxidase activity in the culture medium. This effect can be explained by a dose-dependent increase of oxidative stress, which causes decreased activity of antioxidant enzymes (**Nel *et al.*, 2006**).

Table 4 The effect of vermiculite nanoparticles on antioxidant enzyme activity in the culture medium of *B. subtilis* IMV V-7023

Content of vermiculite nanoparticles, g/L	Catalase activity (mmol H_2O_2/min per mg protein)	Peroxidase activity (mmol indigo carmine/min per mg protein)
0	**6.98±0.12**	**0.75±0.03**
1.5	**7.61±0.17**	**1.92±0.89**
2.5	**7.69±0.17**	**2.09±0.95**
5.0	**7.79±0.18**	**1.61±0.65**

Creating a free-flowing complex bacterial preparation

Consequently, the vermiculite had a stimulating effect on physiological and biochemical activity of investigated strains of bacteria. Also, this mineral was characterized by considerable moisture capacity. We found that at 60 % moisture capacity (optimum for growth of microorganisms), 100 g of vermiculite were keeping to 300 mL of the culture medium required for reproduction of bacteria. According to the results of interaction the strains *A. vinelandii* IMV V-7076 (**Patent № 72856**) and *B. subtilis* IMV V-7023 (**Patent № 54923A**) with particles of exfoliated vermiculite, the free-flowing complex bacterial preparation for crop production was created.

Creating a free-flowing complex bacterial preparation requires optimization of the culture conditions of production (**Kameneva, 2009**).We has optimized the composition of culture media for the cultivation of individual bacterial monoculture and mixed culture of bacteria which are components of the complex preparation. It was established the best medium for growth of mixed culture of bacteria was a liquid culture medium of such composition (g/L): treacle 30.0, corn-steep extract 2.0, $K_2HPO_4 \cdot 3H_2O$ 0.25, KH_2PO_4 0.25, $MgSO_4 \cdot 7H_2O$ 0.3, NaCl 0.3, $CaCO_3$ 3.0 (pH 7.0-7.2). The cultivation of mixed cultures of *A. vinelandii* IMV V-7076 and *B. subtilis* IMV V-7023 in this medium for 24 hours allowed getting in culture liquid more than 10^9 cells/mL of each strain.

The manufacturing process of granular complex bacterial preparation on the basis of high active strains *A. vinelandii* IMV V-7076 and *B. subtilis* IMV V-7023 was optimized. Because the bacilli were characterized higher specific growth rate, compared with *A. vinelandii* IMV V-7076, the inoculation vermiculite mixed suspension of bacteria in optimized nutrient medium was amounted about 10^7 CFU/mL of *A. vinelandii* IMV V-7076 and 10^6 CFU/mL of *B. subtilis* IMV V-7023 in the mass correlation to this carrier 3:1. Thus, the incubation with vermiculite for 24 h at 28 ^oC provided obtaining the high-quality of complex bacterial preparation containing in each its gram more than 10^9 CFU of *A. vinelandii* IMV V-7076 and *B. subtilis* IMV V-7023 (Table 5).

Table 5 The number of *A. vinelandii* IMV V-7076 and *B. subtilis* IMV V-7023 in the powdered vermiculite preparation during the periodic of incubation

Type of bacteria in the preparation	Number of bacteria (cells/g) in the preparation during the incubation, days		
	0	1	2
A. vinelandii	$(4.0\pm0.1)\cdot10^7$	$(1.9\pm0.1)\cdot10^9$	$(1.7\pm0.1)\cdot10^9$
B. subtilis	$(1.4\pm0.1)\cdot10^7$	$(3.8\pm0.2)\cdot10^9$	$(1.0\pm0.1)\cdot10^{10}$
A. vinelandii	$(3.7\pm0.2)\cdot10^7$	$(1.6\pm0.1)\cdot10^9$	$(1.8\pm0.1)\cdot10^9$
B. subtilis	$(1.9\pm0.1)\cdot10^6$	$(1.1\pm0.1)\cdot10^9$	$(3.7\pm0.4)\cdot10^9$
A. vinelandii	$(3.8\pm0.3)\cdot10^7$	$(2.6\pm0.2)\cdot10^9$	$(2.1\pm0.1)\cdot10^9$
B. subtilis	$(1.2\pm0.1)\cdot10^5$	$(3.0\pm0.1)\cdot10^8$	$(6.0\pm0.2)\cdot10^8$

Therefore, it has been optimized the process of making free-flowing complex bacterial preparation for crop growing. The technology allows getting more than 10^9 of cells of these bacteria strains per gram of the preparation. The preparation is stable during storage, improves nitrogenous and phosphorus nutrition of plants, stimulates their growth by biologically active substances and protects plants from lesion by phytopathogenic micromicetes and bacteria **(Patent №106135C2).**

Effect of free-flowing complex bacterial preparation on the crop cereals plants

Growing of winter wheat «Tsarivna», whose seed was processed with the free-flowing complex bacterial preparation, was accompanied by an increase in grain yield up to 0.57 – 0.62 t/ha, the content of crude protein and fiber in it, up to 0.6-1.0 % and 1.1 – 1.3 %, respectively (Table 6). The lesion plant by root rot and septoria leaf spot of wheat was significantly decreased.

Seeds treatment of spring barley grade «Nabat» by the free-flowing complex bacterial preparation increased of the yield of grain up to 0.35 – 0.43 t/ha, the content of crude protein in it, up to 0.4 – 0.6 %, reduced lesion plant leaves of dark brown spot (Table 6).

Table 6 Effect of free-flowing complex bacterial preparation on the crop cereals plants

Variant of plants growing	Harvest of winter wheat „Tsarivna"		Harvest of spring barley „Nabat"	
	t/ha	%	t/ha	%
Control (without fertilization)	2.63	100.0	1.87	100.0
Accessory production (Background)	2.91	116.5	2.08	111.2
Background+ bacterial preparation	3.48	131.5/119.9	2.43	130.0/117.0

CONCLUSION

Thus, it has been **developed** the technology of creation of the free-flowing complex bacterial preparation for crop growing. This preparation was stable during storage and convenient for use in agroecosystems cereals. It has been shown the positive impact of application of the free-flowing complex bacterial preparation on the crop seeds of winter wheat and spring barley. The preparation had **the** significantly **increased yield** and improved the quality of grain. It has been determined that in rhizosphere soil of cereals, whose seeds were treated by the complex bacterial preparation**, significant changes** have been **observed in the microbial** coenosis, in which the total number of bacteria, the content of oligotrophic bacteria, phosphate-mobilizing bacteria and some other physiological-trophic groups of microorganisms was increasing.

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