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IMPACT OF A NUMBER OF FACTORS ON PHYSIOLOGICAL AND BIOCHEMICAL ACTIVITY OF STRAINS – COMPONENTS OF AZOGRAN, A COMPLEX BACTERIAL PREPARATION

The use of microbial preparations in plant-growing can be due to the correction of biological processes in agroecosystems and stimulates growth and development of plants. The efficiency of this process is dependent on biotic and abiotic factors, however their influence on introduction microorganisms in phytosphere is insufficiently studied. The article summarizes some results of recent studies, related to the impact of a number of environmental factors on physiological and biochemical activity of nitrogen-fixing bacteria Azotobacter vinelandii IMV B-7076 and phosphate-mobilizing strain Bacillus subtilis IMV B-7023 – components of Azogran, a complex bacterial preparation for plant growing. The dependence of the physiological and biochemical activity of these bacteria, including their antioxidant potential, on biotic and abiotic environmental agents was determined. The impact of a number of factors on chemotaxis, energy metabolism of these bacteria, their synthesis of substances of phenol nature, and other biologically active substances, which may influence the efficiency of using this preparation in plant growing, was studied. Azogran inhibits the spread of phytopathogens and some kinds of phytophages in agroecosystems, is capable of protecting plants from the oxidative stress and enhancing on 16–37 % their crop productivity.

K e y w o r d s: Azotobacter vinelandii, Bacillus subtilis, physiological and biochemical activity, a complex bacterial preparation Azogran.

In recent decades, a number of countries have been paying great attention to the biologization of agriculture. One of the relevant components of this approach is the application of bacterial preparations with the purpose of correcting microbial processes in agroecosystems. It was established that the most efficient way to achieve the mentioned purpose is the application of complex microbial preparations, produced on the basis of two or more strains, which condition synergistic stimulating impact on growth, development, and productivity of crops [1].

On basis the interaction of bentonite particles and highly efficient strains of nitrogen-fixing bacteria *Azotobacter vinelandii* IMV B-7076 [2] and phosphate-mobilizing bacteria *Bacillus subtilis* IMV B-7023 [3] was used by us to elaborate a complex granulated bacterial preparation Azogran. It is remarkable for the stability of its composition at long storage, promotes growth and development of plants, stimulates their growth with biologically active substances of bacterial nature, and protects plants from phytopathogens and phytophages. This preparation enhances crop productivity of vegetables and technical plants by 18–37 % [1].

A free-flowing form of the preparation is more convenient for the inoculation of cereal seeds. Taking it into consideration, we have developed a free-flowing complex preparation for plant growing, based on the interaction of the

mentioned bacterial strains and nanoparticles of expanded vermiculite. The obtained bacterial preparation contains over 10^9 viable cells of the mentioned bacterial strains and is characterized by the stability of its composition at long storage. The application of this preparation in agroecosystems of spring barley and winter wheat increased the crop productivity by 16 and 20 % respectively [4, 5].

It is known that with the introduction of complex microbial preparations into the agroecosystem, the introduced bacteria will interact both among themselves and with the plant, soil biota, and nanoparticles of different nature. The mechanisms of the impact of these factors on bacteria, which are components of the complex preparation for plant growing have not been studied well enough.

This work summarizes some results of the studies on the impact of a number of causative agents, which may have an effect on the bacteria, introduced into the agroecosystem, on the physiological and biochemical activity of *A. vinelandii* IMV B-7076 and *B. subtilis* IMV B-7023 – components of Azogran, a highly efficient complex bacterial preparation. The research on these issues is an urgent task of modern science, which will deepen our knowledge of the properties of bacterial strains that are the basis of the microbial preparation for plant growing, and will disclose the regularities of their interaction with phytosphere components when introduced into the agroecosystem. Further on, it will allow forecasting the efficiency of the application of bacterial preparations in plant growing with the purpose of correcting microbial processes in agroecosystems, depending on the species of plants and the impact of natural environmental factors.

It is known that when bacterial preparations are used in plant-growing, the native microbiota of agroecosystems may cause the opposition to microorganisms, introduced into soil; as a result, their number may decrease by one order in 10 days [6]. It was established by us that the interaction of components of the complex bacterial preparation and some representatives of soil biota may influence the manifestation of their activity on the growth of plants. For instance, the germination of cucumber seeds of Dzherelo cultivar in Knop's medium, containing 10^6 CFU/ml of *B. subtilis* IMV B-7023, was accompanied with the accumulation of indolil acetic acid there and the reduction in plant roots, compared to the control. At the same time, when bacilli were cultivated together with the ciliates of *Colpoda steinii,* capable of consuming these bacteria, the accumulation of this phytohormone in the medium decreased considerably, whereas the morphometric indices of sprouts increased. This fact may be conditioned by the capability of increased concentrations of auxins to inhibit the growth of plant roots [7].

It was demonstrated by us that *B. subtilis* IMV B-7023 is the antagonist of a wide range of phytopathogenic bacteria and micromycetes. The most visible radii of growth inhibition zones, induced by the impact of these bacilli on phytopathogenic bacteria, were established for *Pseudomonas syringae* pv. *syringae* – 8 mm, *Clavibacter michiganensis subsp. michiganensis* 10_2 – 14 mm, *C. michiganensis subsp. michiganensis* 13a – 6 mm, *Xanthomonas campestris* pv. *campestris* 8003b – 19.6 mm [8].

It was determined that the pre-sowing treatment of tomato seeds, Bily nalyv cultivar, with the suspension of a phytopathogen *Clavibacter michiganensis subsp. michiganensis* 10_2 was accompanied with an evident reduction of

plant growth. At the same time, the combined inoculation of the seeds with the phytopathogen and *B. subtilis* IMV B-7023 enhanced its germination by 14.6 % and the growth of plants – by 16–18 %. In addition, when tomato plants and fruit were intentionally infected with this phytopathogen (10^9 col/ml) after four months of their cultivation, when the seeds had been previously treated with *C. michiganensis subsp. michiganensis* 10_2 and *B. subtilis* IMV B-7023 the development of bacterial cancer of these plants was not determined. At the same time when control plants were treated with this phytopathogen (seeds were treated with water), they developed classic symptoms of bacterial cancer [9].

When spring barley seeds of Nabat cultivar were inoculated with this preparation, the damage of its leaves from dark brown spot diseases decreased considerably. When winter wheat seeds were treated with the complex preparation Azogran, the spread of Septoria spot on the spikelet and the development of this disease in plants decreased considerably [5].

It was established by us that the bacteria of *B. subtilis* IMV B-7023 are capable of inhibiting the spread of phytophages in agroecosystems. On the tenth day of greenhouse cultivation, after the epiphytic treatment of *Pelargonium zonale* plants, using these bacteria, the number of imago and larvae of greenhouse whitefly decreased by over 50 % (from 43 per 1 plant down to 20). When coleus plants were similarly treated with this bacterial strain, the number of imago and larvae of this phytophage decreased from 30 per 1 plant in the control down to 18 [10].

After the epiphytic treatment of these plants with the suspension against *Myzodes persicae*, the biological efficiency of the insecticide activity of *B. subtilis* IMV B-7023 was from 40 to 50 %. For instance, on the 10^{th} day after the treatment, the number of this phytophage was 37 per one pelargonium plant whereas there were 55 on the control one. As for *Coleus* plants, there were 22 and 45, respectively. This effect was somewhat conditioned by the proteolytic activity of these bacteria [10].

It is known that the primary stages of the interaction between microorganisms, introduced into the agroecosystem, and a plant, are chemotaxis of their cells to root exudates and their adhesion to the root surface. It was demonstrated by us that the adhesion of *B. subtilis* IMV B-7023 to solid surfaces is greatly dependent on the motility of cells. It was also demonstrated that the cells of bacilli from the exponential phase of growth (24 h of cultivation) adhered to the hydrophilic surfaces in the highest amounts.This is conditioned by high motility of bacteria, which is remarkable for cells in the exponential phase of their growth. At longer cultivation (48–72 h) the motility of bacteria and their adhesion to the surface decreased [11].

The chemotaxis of *B. subtilis* IMV B-7023 and their adhesive properties are greatly conditioned by their combined cultivation with *A. vinelandii* IMV B-7076. It was demonstrated by us that the chemotaxis index of *B. subtilis* IMV B-7023 (the ratio of the number of bacteria in the unit of capillary volume with the attractant, glucose, and the corresponding index in the control) in the pure culture is 7.1, and that of *A. vinelandii* – 3.4. In the mixed culture, this index for bacilli decreased considerably and was 4.3, whereas it almost did not change for *Azotobacter vinelandii* IMV B-7076 – 3.6. In addition, it was demonstrated that the negative charge of *B. subtilis* IMV B-7023 cells

increased considerably in the mixed culture of these bacteria. For instance, if the cells in the pure culture of *Azotobacter* had the charge of –45.6 mV, and bacilli –35.8 mV, these indices in the mixed culture were –46.0 and –45.3 mV respectively [12].

It was established by us that the reason of these differences is the sorption of the polysaccharide complex of *A. vinelandii* IMV B-7076 on the surface of *B. subtilis* IMV B-7023 cells. With the introduction of 0.0005 g/l of polysaccharide complex in the suspension of bacilli, their ζ-potential increased considerably and was –42.0 mV. The sorption of this complex on the surface of *B. subtilis* IMV B-7023 cells is likely to condition the blocking of chemotaxis receptors in these bacteria [12], and possibly that of the adhesins of the surface of bacilli.

When bacteria are introduced into the agroecosystem in the composition of the preparation, their cells will interact with a considerable number of natural nanoparticles, which may condition a considerable impact on physiological and biochemical properties of microbial populations. It was demonstrated by us that the interaction of microorganisms – components of a complex bacterial preparation – with nanoparticles of vermiculite has a significant impact on energetic processes in cells. For instance, when *B. subtilis* IMV B-7023 were cultivated in the medium, containing 0.5 g/l of particles of this mineral, the dehydrogenase activity of bacteria increased by 34 %. With its higher content in the medium (1.0–5.0 g/l) this index increased by 21–28 % [13].

At the same time, when *A. vinelandii* IMV B-7076 were cultivated with nanoparticles of this mineral, the dehydrogenase activity increased with the increase of its content in the medium. With the introduction of 5 g/l of vermiculite this activity of bacteria increased by 40 % compared to the control [13]. This impact of vermiculite on the dehydrogenase activity of the mentioned bacteria may be conditioned by the increase in the mass transfer of oxygen, when they are cultivated with disperse materials and by the contact interaction of cells and nanoparticles, which influences the physiological and biochemical activity of microorganisms [14].

It was established that the dehydrogenase activity of *B. subtilis* IMV B-7023 depends on a number of physical and chemical environmental factors. It was determined that it has the highest values at the temperature of 37 °C in the medium, containing 10 g/l of glucose and has pH 7.0. A considerable stimulating impact on this activity was observed at the action of Mg^{2+} and Ca^{2+} ions. The introduction of 0.25 and 1.0 mM of magnesium ions into the medium in the form of $MgCl_2$ was accompanied by the increase in dehydrogenase activity of this strain, compared to the control, by 23 and 43 % respectively. However, the stimulating impact of this cation decreased with further increase in its content in the medium [15].

The cultivation of this strain in the medium with calcium cations had even greater impact on the dehydrogenase activity. The index of this activity is increased by 29 % in the presence of 0.25 mM Ca^{2+} in the medium. The increase in the concentration of this ion to 0.5 and 1.0 mM was accompanied by the increase in the dehydrogenase activity of *B. subtilis* IMV B-7023 by 61 and 83 %. Its highest indices were determined in the presence of 10 mM Ca^{2+} in the medium. Further increase in its concentration was accompanied by the decrease in the stimulating activity of this cation. At the same time, the intro-

duction of ferric iron ions in the form of $FeCl_3$ conditioned a negative impact on the dehydrogenase activity of these bacteria [15].

It is known that in natural conditions plants are exposed to a negative impact of environmental factors, resulting in the generation of reactive oxygen intermediates in cells, which may condition the oxidative stress. The strains of bacteria, applied in plant growing in the form of microbial preparations, are capable of mitigating the impact of negative factors on the growth and development of plants. It was established by us that bacteria – components of a complex bacterial preparation Azogran – condition a considerable antioxidant impact on plant seeds, exposed to oxidative stress via their previous treatment with hydrogen peroxide. After treating the seeds of vetch, Margarita cultivar, with the suspension of these bacteria, the germination of the latter and the number of normally formed sprouts increased compared to the control (seeds, treated with hydrogen peroxide) by 51.9 and 58.6 % respectively. The inoculation of these seeds was accompanied by the decrease in the infection of sprouts with micromycete almost by 65.0 % [8].

It was demonstrated that *B. subtilis* IMV B-7023 is characterized by the reducing ability, the antiradical activity with respect to OH, and also 2.2'-diphenyl-1-picrylhydrazyl free radical. These indices can play an important role in the protection of the strain from reactive oxygen intermediates, and they are capable of influencing the antioxidant properties of a vegetable organism [16].

It was found that if introduced into the nutrient medium with *B. subtilis* IMV B-7023 of 0.05–1.0 g/l nano-SiO_2 the oxidant activity of the culture medium (CM) of bacilli increased by 43–60 % and the antioxidant activity decreased by 4.5–11.8 %.

SiO_2 nanoparticles had a different effect on the antiradical activity of CM of *B. subtilis* IMV B-7023. In particular, nano-SiO_2 had no significant effect on the ability of the CM of bacilli to inactivate the 2.2'-diphenyl-1-picrylhydrazyl free radical. However, in the presence of nanomaterial of 0.01–1.0 g/l hydroxyl radical scavenging in the CM of this bacteria decreased by 7.2–17.6 % compared to the control. Low doses of silica nanoparticles stimulated the reducing power of the CM of bacteria [17].

It was established by us that nanoparticles of natural minerals condition a considerable impact on the activity of the enzymes of the antioxidant protection of *B. subtilis* IMV B-7023. For instance, when cultivated in the medium, containing 0.05–0.5 g/l nanoparticles of SiO_2 or 1.5–2.5 g/l of vermiculite the activity of the extracellular peroxidase increased considerably. However, this index decreased with further increase in the content of these nanomaterials in the medium. These nanomaterials had no considerable impact on the catalase activity or the intracellular peroxidase activity of these bacteria [18].

It was determined that *B. subtilis* IMV B-7023 bacteria are capable of accumulating the compounds of phenol nature in the nutrient medium, which, according to the literature data, have a wide spectrum of biological activity and are characterized by a high antioxidant potential [19]. These bacteria accumulated the most evident number of these compounds in the gelatin-containing medium – up to 94 μg/ml. In the mineral medium with calcium orthophosphate and glucose, the content of these compounds reached 58.3 μg/ml, in the nutrient medium with calcium glycerophosphate and glucose – 27 μg/ml and in the same nutrient medium without glucose – 10.2 μg/ml. The results obtained

testify to a considerable role of the cultivation conditions of these bacteria on their accumulation of compounds of phenol nature [20].

In addition, this strain produces a number of free aminoacids, the quality and quantity composition of which depends on cultivation conditions. The capability of these bacteria to produce methionine, lysine, and histidine is of special interest [8], as these aminoacids can neutralize high reactive free radicals of oxygen, capable of damaging proteins, lipids, and nucleic acids [21].

Therefore, the results of these studies demonstrate that the physiological and biochemical activity of *B. subtilis* IMV B-7023 *and A. vinelandii* IMV B-7076 – components of a complex bacterial preparation Azogran, which may be used in plant growing in a granulated, free-flowing, and suspension form, depends on a considerable number of factors, including the impact of natural nanomaterials. This preparation is a highly efficient means of improving the growth, development, and crop productivity of plants due to the complex impact of bacteria on the components of agroecosystems. These bacteria synthesize phenol compounds, aminoacids, and other biologically active substances, mitigate the negative impact of reactive oxygen intermediates on plants, inhibit phytopathogenic microorganisms and phytophages in agroecosystems.

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ВПЛИВ РЯДУ ФАКТОРІВ НА ФІЗІОЛОГО-БІОХІМІЧНУ АКТИВНІСТЬ ШТАМІВ – КОМПОНЕНТІВ КОМПЛЕКСНОГО БАКТЕРІАЛЬНОГО ПРЕПАРАТУ АЗОГРАН

Резюме

Застосування мікробних препаратів у рослинництві дозволяє корегувати біологічні процеси в агроекосистемах та покращувати ріст і розвиток рослин. Ефективність цього процесу залежить від ряду біотичних і абіотичних факторів, вплив яких на інтродуковані у фітосферу мікроорганізми недостатньо досліджений. У статті узагальнені деякі результати досліджень останніх років, що стосуються впливу ряду факторів середовища на фізіолого-біохімічну активність азотфіксуючих бактерій *Azotobacter vinelandii* IMB B-7076 та фосфатмобілізуючого штаму *Bacillus subtilis* IMB B-7023 – компонентів комплексного бактеріального препарату Азогран для рослинництва. Визначена залежність фізіолого-біохімічної активності цих бактерій, у тому числі їх антиоксидантного потенціалу, від біотичних та абіотичних чинників середовища. Досліджена дія ряду факторів на хемотаксис, енергетичний метаболізм даних бактерій, синтез ними речовин фенольної природи та інших біологічно активних сполук, які можуть впливати на ефективність застосування даного препарату в рослинництві. Азогран пригнічує поширення в агроекосистемах фітопатогенів та деяких видів фітофагів, здатен захищати рослини від оксидативного стресу, підвищує їх урожайність на 16–37 %.

Ключові слова: Azotobacter vinelandii, Bacillus subtilis, фізіолого-біохімічна активність, комплексний бактеріальний препарат Азогран.

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ВЛИЯНИЕ РЯДА ФАКТОРОВ НА ФИЗИОЛОГО-БИОХИМИЧЕСКУЮ АКТИВНОСТЬ ШТАММОВ – КОМПОНЕНТОВ КОМПЛЕКСНОГО БАКТЕРИАЛЬНОГО ПРЕПАРАТА АЗОГРАН

Резюме

Применение микробных препаратов в растениеводстве позволяет корригировать биологические процессы в агроэкосистемах и улучшать рост и развитие растений. Эффективность этого процесса зависит от ряда биотических и абиотических факторов, влияние которых на интродуцированные в фитосферу микроорганизмы недостаточно исследовано. В статье обобщены некоторые результаты исследований последних лет, которые касаются влияния ряда факторов на физиолого-биохимическую активность азотфиксирующих бактерий *Azotobacter vinelandii* IMB B-7076 и фосфатмобилизирующего штамма *Bacillus subtilis* IMB B-7023 – компонентов комплексного бактериального препарата Азогран для растениеводства. Определена зависимость физиолого-биохимической активности этих бактерий, в том числе их антиоксидантного потенциала, от биотических и абиотических факторов среды. Исследовано воздействие ряда факторов на хемотаксис, энергетический метаболизм этих бактерий, синтез ими вещества фенольной природы и других биологически активных соединений, которые могут влиять на эффективность применения данного препарата в растениеводстве. Азогран угнетает распространение в агроэкосистемах фитопатогенов и некоторых видов фитофагов, способен защищать растения от оксидативного стресса, повышает их урожайность на 16–37 %.

Ключевые слова: Azotobacter vinelandii, Bacillus subtilis, физиолого-биохимическая активность, комплексный бактериальный препарат Азогран.

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