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**INFLUENCE OF PLANT GROWTH REGULATORS ON RESISTANCE OF
LELLIOTTIA NIMIPRESSURALIS CCM 32-3 TO GRAIN PROTECTANTS**

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Summary. *The aim of our research was to study the effect of plant growth regulator “Biolan” on the activity of exopolysaccharides (EPS) production by the bacterium Lelliottia nimipressuralis CCM 32-3 and its resistance to some seed dressing agents. The objects of the research were bacteria L. nimipressuralis CCM 32-3, plant growth regulator (PGR) “Biolan”, seed dressing agents of cereal plants. To study the quantitative content of exopolysaccharides in the culture fluid of L. nimipressuralis CCM 32-3, the phenol-sulfuric method was used. Determination of resistance of L. nimipressuralis CCM 32-3 cells to seed dressing agents was carried out on the MPA medium (by the method of wells) under conditions of deep cultivation. Studies have been carried out to quantify the exopolysaccharides in the culture fluid of the bacterium L. nimipressuralis CCM 32-3. It was found that one-day culture contains 22.8 mg/l EPS; two days later their number was 35.8 mg/l. The positive effect of PGR “Biolan” on the amount of EPS was revealed. The addition of this preparation to the nutrient medium contributed to an increase in the number of EPS by 20.6 % in one-day culture of L. nimipressuralis CCM 32-3 and by 17.9 % in two-day one. The action of fungicides (“Vitavax 200 FF”, “Lamardor FS 400”, “Raxil Extra”), which were applied according to the manufacturer’s guidelines, on the growth and cell viability of L. nimipressuralis CCM 32-3 has been studied. The bacteriostatic effect of the studied seed dressing agents on the viability of the strain cells was revealed: there was a decrease in cell titer compared to control after the “Vitavax 200 FF”, “Raxil Extra”, and “Lamardor FS 400” application by 21 %, 9 %, and 25 %, respectively. It was found that the introduction of “Biolan” into the nutrient medium neutralizes the negative effect of “Vitavax 200 FF” and “Raxil Extra”, however, it did not have any similar effect on “Lamardor FS 400”. The positive action of “Biolan” on L. nimipressuralis CCM 32-3 was established. The addition of PGR to the culture medium for cultivating the strain contributed to an increase in the content of EPS, which increased the resistance of cells to the studied seed dressing agents.*

Keywords: *exopolysaccharides, Lelliottia nimipressuralis CCM 32-3, seed protectants, plant growth regulator “Biolan”.*

Introduction

The use of microbial preparations to optimize the nutrition of agricultural plants, improve their growth and increase productivity is one of the aspects of biological agriculture [1–3]. Effective strains of soil bacteria were identified in the Department of Microbiology of the FSBSI “Research Institute of Agriculture of Crimea”. Based on these strains, modern microbial preparations were created including one based on *L. nimipressuralis* CCM 32-3 [4]. Some researchers add polysaccharides of other microorganisms to the composition of biological products to improve their properties [5]. It is proved that biopreparations containing exopolysaccharides (EPS) have a longer shelf life, improve sowing qualities and stimulate plant growth [6]. In our earlier studies, we indicated the ability of bacteria *L. nimipressuralis* 32-3 CCM to synthesize EPS [7].

Exopolysaccharides are high-molecular exogenous metabolic products of microorganisms. Bacterial EPS are biologically active substances with antiviral and bactericidal effects. They enhance the medicinal effect of antibiotics by stimulating factors

of nonspecific immunity of microorganisms. EPS act as intermediaries in the interaction of bacteria-producers of EPS with other macro-and microorganisms [8, 9].

Moreover, it was experimentally established that rhizobial EPS have an impact on the number of nodules formed on the roots of legumes and their nitrogen-fixing activity. They play a multifunctional role in the formation of bean-rhizobial symbiosis [10–15]. Currently, bacterial EPS are widely used in many industries: textile, food, pharmaceutical, chemical, oil, as well as in medicine and agriculture.

Taking into account the broad spectrum of EPS and their effect on the adaptive characteristics of the organisms, the **aim of our research** was to study the effect of plant growth regulator “Biolan” on the ability of *L. nimipressuralis* CCM 32-3 to synthesize these compounds, as well as resistance to some seed dressing agents.

Materials and methods

Quantitative determination of EPS in the culture liquid of the bacteria *L. nimipressuralis* CCM 32-3 was carried out by the phenol-sulfuric method in the laboratory. *L. nimipressuralis* CCM 32-3 strain was cultured on the meat-and-peptone agar (MPA) medium for 1-2 days. We used pure MPA medium and MPA with plant growth regulator (PGR) “Biolan” at a concentration of 10^{-7} mg/l. “Biolan” is a broad-spectrum plant growth biostimulator obtained during the biotechnological cultivation of epiphyte fungi isolated from the root system of medicinal plants [14]. Bacteria biomass was washed off with saline solution (0.85 % NaCl), the cell suspension was centrifuged for 20 minutes at 6,000 rpm. Then, 0.05 ml of 80 % phenol was added to 2 ml of fugate. After that, 5 ml of concentrated H_2SO_4 was poured, the tubes were stirred and kept for 10 minutes. Then, the tubes were shaken and placed for 15 minutes in a water bath (25–30 °C). The intensity of the yellow-orange color was measured on a photoelectric colorimeter at a wavelength of 490 nm [15].

Determination of *L. nimipressuralis* CCM 32-3 cells resistance to modern seed dressing agents (according to the technologies of growing agricultural plants) was performed by the method of holes on MPA medium [16]. The following seed dressing agents were used: “Vitavax 200FF”, “Lamardor FS 400”, “Raxil Extra”. They were diluted in the recommended doses – 2.5 l, 0.2 l, and 2.0 l, respectively, in 10 l of water.

To determine the effect of seed dressing agents on the growth of *L. nimipressuralis* CCM 32-3 under conditions of deep cultivation, the strain was grown on the liquid glucose-asparagine medium for one day at a temperature of 28 °C on the rockers (UVMT 12-250) with rotation speed 230 rpm. The studied protectants and PGR “Biolan” were added to the flasks with the nutrient medium simultaneously with the biomass of the bacteria. The titer of the culture was determined by seeding certain dilutions of its suspension on MPA and subsequent accounting of colony-forming units (CFU) grown on the medium after 3 days.

Statistical analyses of the research results were conducted according to the methodology of B. A. Dospekhov [17].

Results and discussion

The results indicate that *L. nimipressuralis* CCM 32-3 in the process of life produces substances belonging to EPS. The quantitative determination of EPS showed that the culture liquid of one-day culture of *L. nimipressuralis* CCM 32-3 grown on MPA contains 22.8 mg/l of exopolysaccharides. After two days of cultivation, the quantity of EPS increased by 13 mg/l (57 %) and was 35.8 mg/l (figure 1).

Since the technology of production of the biological preparation involves the cultivation of *L. nimipressuralis* CCM 32-3 in a liquid medium for two days, further determination of the number of EPS, in our opinion, was impractical.

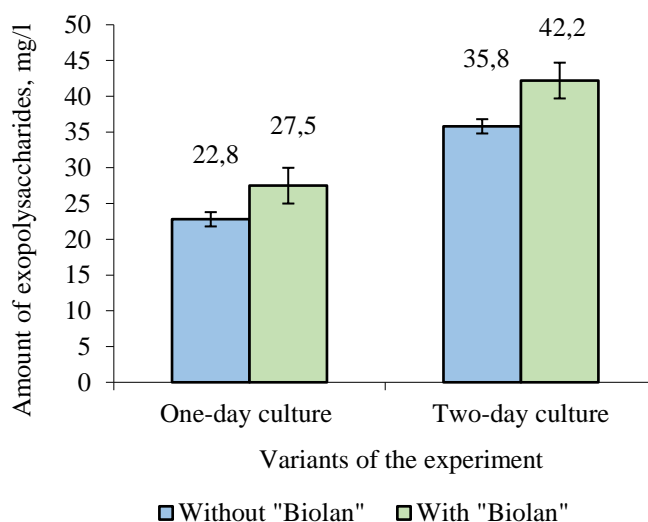


Figure 1 – Effect of PGR “Biolan” on the activity of the production of EPS by the bacteria *L. nimipressuralis* CCM 32-3

Our previous studies have shown a positive effect of PGR “Biolan” on biomass productivity and alkaline phosphatase activity, as well as an increase in the synthesis of phytohormones by the bacteria *L. nimipressuralis* CCM 32-3 [18, 19]. Hence, the study of the effect of plant growth regulator “Biolan” on the synthesis of EPS by the bacteria *L. nimipressuralis* CCM 32-3 was of particular interest. Thus, the results show that its addition to the nutrient medium contributed to an increase in the content of EPS by 20.6 % (compared to the data obtained after the cultivation of the strain on a pure medium of MPA without “Biolan” addition) in one-day culture and 17.9 % – in two-day one.

According to literature sources, it is known that EPS form a dense layer on the surface of the cell wall of bacteria, protecting them from the effects of stress factors: high and low pH, high temperature, drying, freezing, action of detergents and heavy metals [20–23]. Furthermore, some scientists have shown that *Paenibacillus polymyxa* EPS stimulate growth, development and protective reactions of wheat [24, 25].

We have conducted experiments in which investigated the resistance of cells *L. nimipressuralis* 32-3 CCM to seed dressing agents: “Vitavax 200 FF”, “Lamardor FS 400”, “Raxil Extra”. The results of the experiments indicated the resistance of the cells to the studied fungicides. It was found that around the holes in which dressing agent was introduced, there were no zones of inhibition of bacterial growth. We observed the growth of this strain in the form of a continuous lawn.

Studies have shown that chemical dressing agents (“Vitavax 200 FF”, “Lamardor FS 400”, “Raxil Extra”), if applied according to the manufacturer’s guidelines, visually did not inhibit *L. nimipressuralis* 32-3 CCM growth in laboratory experiments.

However, the impact of seed dressing agents on the viability of *L. nimipressuralis* CCM 32-3 cells under submerged cultivation differentiated more clearly. Thus, our studies have shown that the addition of “Vitavax 200 FF” to the nutrient medium reduced the titer of viable cells in one-day culture of the CCM 32-3 by 21 % compared to the control: from 0.65 billion CFU/ml to 0.51 billion CFU/ml (figure 2).

It is possible to weaken the bacteriostatic effect “Vitavax 200 FF” due to the PGR “Biolan”. Its addition to the culture medium contributed to an increase in the titer of viable cells not only in pure culture (by 15 % compared to control) but also in culture with the added dressing agent (by 33 %). The titer of viable cells in one-day culture with the combined use of “Biolan” and “Vitavax 200 FF” was even slightly higher than the control values (by 4.6 %).

Studying the influence “Raxil Extra” on the viability of *L. nimipressuralis* CCM 32-3 cells, similar results were received. Adding it to the culture medium also showed the bacteriostatic effect, but slightly less than with “Vitavax 200 FF”.

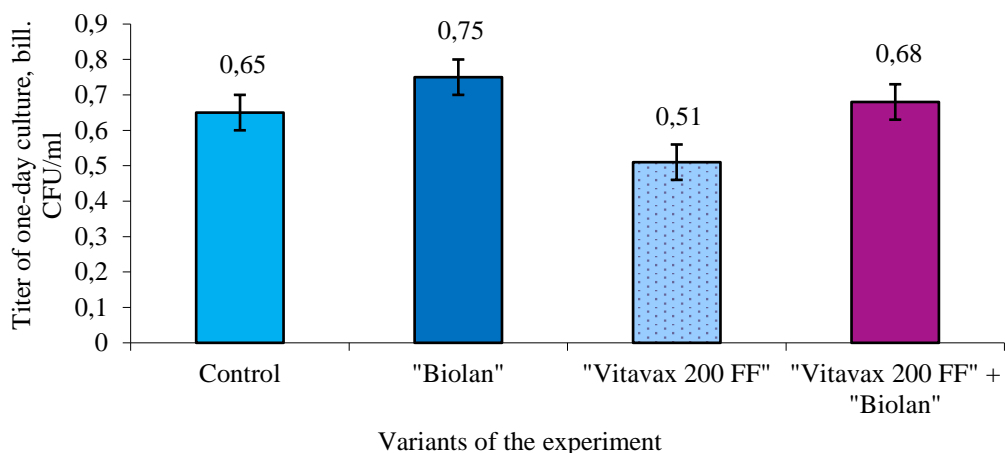


Figure 2 – Influence of PGR “Biolan” and seed dressing agent “Vitavax 200 FF” on the titer of one-day culture of *L. nimipressuralis* CCM 32-3

Thus, the titer of viable cells decreased to 0.59 billion CFU/ml compared to 0.65 billion CFU/ml in control. In this case, the addition of plant growth regulator “Biolan” in the culture medium neutralized the negative effect of the seed dressing agent “Raxil Extra”: titer increased to 0.71 billion CFU/ml, which exceeded the control indicators by 9.2 % (figure 3).

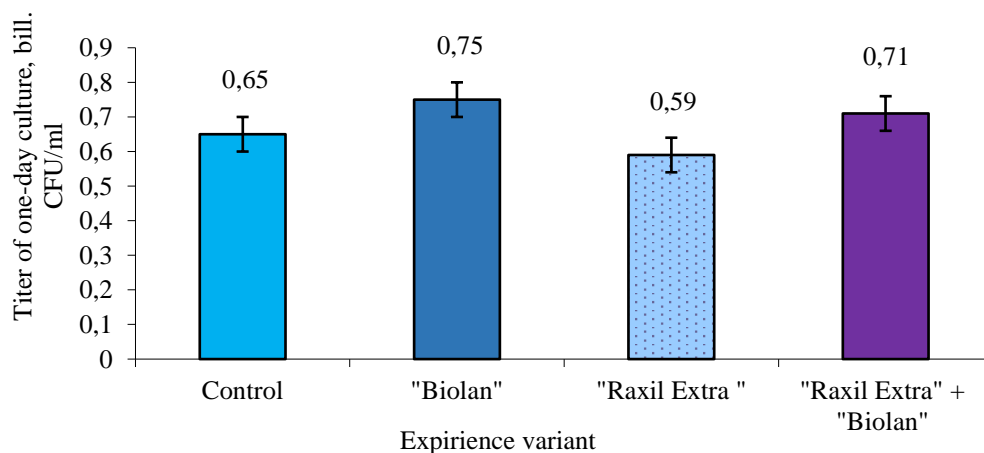


Figure 3 – Influence of PGR “Biolan” and seed dressing agent “Raxil Extra” on the titer of one-day culture of *L. nimipressuralis* CCM 32-3

The study of the influence of “Lamardor FS 400” under the conditions of deep cell cultivation revealed the difference of its action on the viability of *L. nimipressuralis* CCM 32-3 cells compared to the previous two fungicides (figure 4). According to our data, “Lamardor FS 400” had the most depressing effect on the titer of one-day culture: it decreased to 0.49 billion CFU/ml. Even the addition of “Biolan” did not contribute to the improvement of the situation: the titer increased to 0.53 billion CFU/ml, which was less than the control by 0.12 billion CFU/ml (18 %).

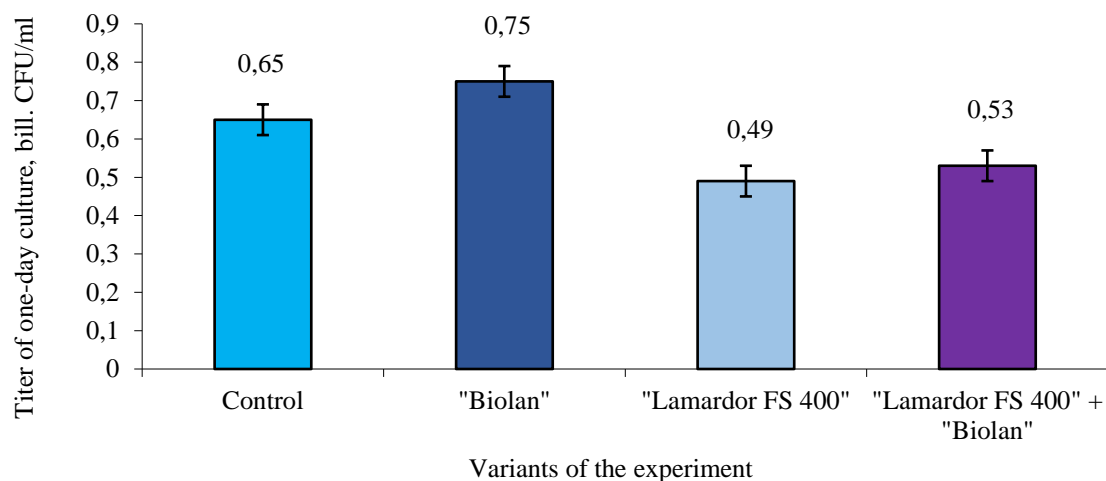


Figure 4 – Influence of PGR “Biolan” and seed dressing agent “Lamardor FS 400” on the titer of one-day culture of *L. nimipressuralis* CCM 32-3

In sum, under conditions of laboratory experiments, we studied the effect of fungicides (“Vitavax 200 FF”, “Raxil Extra”, “Lamardor FS 400”; applied according to the manufacturer’s guidelines) on the growth and viability of *L. nimipressuralis* CCM 32-3 cells. The bacteriostatic effect of the studied seed dressing agents on the viability of the strain cells was revealed: there was a decrease in cell titer compared to control after the application “Vitavax 200 FF”, “Raxil Extra”, and “Lamardor FS 400” by 21 %, 9 %, and 25 %, respectively. It was found that the introduction of “Biolan” into the nutrient medium neutralizes the negative effect of “Vitavax 200 FF” and “Raxil Extra”. However, it did not have same effect on “Lamardor FS 400”.

Conclusions

Strain *L. nimipressuralis* CCM 32-3 in the process of vital activity produces substances belonging to the EPS. The quantitative determination of EPS showed that the culture liquid of one-day culture of *L. nimipressuralis* CCM 32-3 grown on MPA contains 22.8 mg/l of exopolysaccharides. After two days of cultivation, the quantity of EPS increased by 13 mg/l (57 %) and was 35.8 mg/l. The addition of PGR “Biolan” to the nutrient medium contributed to an increase in the quantity of EPS by 20.6 % in one-day culture of *L. nimipressuralis* CCM 32-3 and by 17.9 % in two-day one.

Bacteriostatic effect of fungicides “Vitavax 200 FF”, “Raxil Extra”, “Lamardor FS 400” on cell viability of *L. nimipressuralis* CCM 32-3 was revealed. We observed the reduction of cell titer compared to control after the application “Vitavax 200 FF”, “Raxil Extra”, and “Lamardor FS 400” by 21 %, 9 %, and 25 %, respectively. It was found that the introduction of “Biolan” into the nutrient medium contributes to the intensification of the synthesis of exopolysaccharides EPS by the bacterium, which, in turn, neutralizes the negative effect of “Vitavax 200 FF” and “Raxil Extra”, however, it did not have any similar effect on “Lamardor FS 400”.

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**ВЛИЯНИЕ РЕГУЛЯТОРА РОСТА РАСТЕНИЙ НА РЕЗИСТЕНТНОСТЬ
LELLIOTTIA NIMIPRESSURALIS ССМ 32-3 К ПРОТРАВИТЕЛЯМ ЗЕРНА**

Реферат. Цель наших исследований заключалась в изучении воздействия регулятора роста растений «Биолан» на активность продуцирования экзополисахаридов (ЭПС) бактерией *Lelliottia nimipressuralis* ССМ 32-3 и ее резистентность к некоторым протравителям семян. Объектом исследований были бактерии *L. nimipressuralis* ССМ 32-3, регулятор роста растений (РРР) «Биолан», протравители семян злаковых растений. Для исследования количественного содержания ЭПС в культуральной жидкости бактерии *L. nimipressuralis* ССМ 32-3 использован фенольно-серный метод. Определение резистентности клеток *L. nimipressuralis* ССМ 32-3 к протравителям проводили на среде МПА (методом лунок), а также в условиях глубинного культивирования. Установлено, что односуточная культура содержит 22,8 мг/л ЭПС, а через двое суток культивирования их количество составило 35,8 мг/л. Показано, что добавление в питательную среду РРР «Биолана» увеличивает количество ЭПС, продуцируемых *L. nimipressuralis* ССМ 32-3: на 20,6 % в суточной культуре и на 17,9 % – в двухсуточной. Исследовано действие рекомендованных в производстве доз фунгицидов («Витавакс 200», ФФ, «Ламадор FS», 400, «Раксил Ультра») на рост и жизнеспособность клеток *L. nimipressuralis* ССМ 32-3. Выявлено бактериостатическое действие изученных протравителей на жизнеспособность клеток штамма: титр клеток по сравнению с контролем снижался на 21 %, 9 % и 25 % при введении в питательную среду «Витавакса 200», ФФ, «Раксила Ультра», «Ламадора FS», 400 соответственно. Установлено, что внесение в питательную среду «Биолана» нивелирует негативное действие «Витавакса 200», ФФ и «Раксила Ультра», однако не влияет на воздействие «Ламадора FS», 400. Установлено положительное действие «Биолана» на *L. nimipressuralis* ССМ 32-3. Добавление РРР в питательную среду для культивирования штамма способствовало увеличению содержания ЭПС, что повысило резистентность клеток к исследуемым протравителям семян.

Ключевые слова: экзополисахариды, *Lelliottia nimipressuralis* ССМ 32-3, протравители семян, регулятор роста растений «Биолан».

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