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SELECTION OF MICROORGANISM STRAINS TO PROTECT GRAIN PLANTS FROM FUNGI OF GENUS FUSARIUM

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Abstract. Currently, due to climate change and a number of unfavorable environmental conditions, there is a tendency to decrease the global production of wheat – one of the main grain crops. It is undeniable that the increase in grain production is closely related to the effectiveness of controlling one of the most harmful diseases of grain crops – *Fusarium* wilt, since there are no wheat varieties in the world that are completely resistance to *Fusarium* wilt. The development of biotechnological approaches to obtaining new microbial preparations to protect winter wheat from *Fusarium* pathogens is relevant for the biological protection of winter wheat in organic farming technologies and in integrated protection systems, significantly reducing the xenobiotic pressure on agrocenoses. The purpose of the work was to isolate and screen strains of bacteria of the genera *Bacillus* and *Pseudomonas*, active against mycopathogens of the genus *Fusarium*, detected in seed material of winter wheat. Infection with mycopathogens of the genus *Fusarium* depended on the field resistance of the sort, was quite high and amounted to more than 75% of cases. The main causative agent of *Fusarium* wilt was *F. graminearum*, but *F. oxysporum* and *F. proliferatum* were also isolated. 79 strains of *Bacillus spp.* and 34 strains of *Pseudomonas spp.* were isolated from natural sources. The best antagonists to all isolated *Fusarium* strains were *Bacillus spp.* R14, R31, S19 and *Pseudomonas spp.* WR5 and WR7. Methanolic extracts of secondary exometabolites of the studied strains showed 1,5-2 times higher activity against *Fusarium spp*. when the producers were cultivated on organic nutrient media. Minimum inhibitory concentrations of extracted metabolites of *Bacillus spp.* R14, S19 were determined in the range of 1–4 mg/cm^3, Pseudomonas sp. WR5 – in the range of 2–4 mg/cm^3.

Keywords: winter wheat, mycopathogens of the genus Fusarium, antagonistic bacteria, exometabolites.

Introduction. Formulation of the problem

The yield and quality of wheat seeds depends on many factors, among which a significant role belongs to grain diseases, which limit the potential productivity of winter wheat varieties [1,2]. *Fusarium* wilt is especially damaging. Infection of wheat grain with mycopathogens of the genus *Fusarium* leads to a decrease in yield, reduces germination of seeds, and worsens the quality of bread. Due to the widespread distribution of *Fusarium* wilt of grain crops throughout the world and the danger it poses, the issue of combating its pathogens is acute. The approach in the fight against the pathogens of this disease should be comprehensive, starting from the protection of the seeds in the soil, to several treatments during the growing season. The development and implementation of biological technics of combating *Fusarium* is attractive and promising both from the point of view of cost and from the point of view of environmental safety [3,4]. One of the possible candidates in the fight against and prevention of *Fusarium* pathogens are representatives of the genera *Bacillus* and *Pseudomonas*, the arsenal of secondary metabolites of which is quite large and diverse [5-8].

Analysis of recent research and publications

In today's conditions, winter wheat determines the food security of our country and is the dominant grain crop in agricultural production, which is why increasing the efficiency of the grain complex is one of the global directives for the development of the agricultural sector of Ukraine [7]. In the 21st century, the main problem of agricultural production was the contradiction between modern ecological requirements and the active implementation of intensive crop cultivation technologies. Modern intensive technologies for growing winter wheat should have not only an economic, but also an ecological justification and be maximally adapted to the ecological, soil and climatic conditions of cultivation [9].

Winter wheat is sensitive to pathogens of various diseases, and infection can occur from sowing to harvesting, which leads to a significant decrease in the amount of the crop and deterioration of its quality [10].

The causative agents of many grain crops, including wheat, are fungi, bacteria, and viruses. They enter the plant through soil, seed material, and plant debris that left in the field. However, the most common and dangerous disease of winter wheat is *Fusarium* wilt [11]. Imperfect control of *Fusarium*, which cause a number of diseases, in modern intensive agricultural technologies of grain crops is one of the unsolved problems in the protection of winter wheat plants. The disease is caused by several species of genus *Fusarium*, but the main species is *Fusarium graminearum*. This disease reduces grain yield and quality, and can also contaminate grain with mycotoxins. After the plant infected with the causative agent of *Fusarium* wilt, the fight against this pathogen is ineffective and practically impossible. Therefore, all methods of combating *Fusarium* are preventive, which is especially important in areas with a humid climate and in areas of risky agriculture [12].

The protection of grain crops against *Fusarium* wilt with the biological agents has been actively used since the mid-1990s of the last century. The number of biological agents for such control is currently quite limited, but they can be very effective in reducing the level of disease caused by fungi of the genus *Fusarium*. Public recognition, environmental friendliness, compatibility with chemical means of combating agricultural diseases, duration of action and stability – all these signs indicate the importance of developing tactics for biological control of *Fusarium* wilt. The tools of such biological control can play the most important role in the organic grain industry of the state [6].

At the present time, significant progress has already been achieved in the field of biological control of *Fusarium* wilt. For example, some strains of spore-forming bacteria (*Bacillus spp*.) and yeasts (*Cryptococcus spp*.) exhibit properties that are essential to combat *Fusarium* wilt and to reduce of fungal mycotoxin contamination [13].

Thus, a team of researchers proposed microbial compositions for biological control of *Fusarium* wilt and provided methods of using these compositions to prevent the occurrence, inhibit development, and treat diseases of agricultural plants caused by *Fusarium spp*., and also for good crop storage [14].

According to Krutyakova V.I. with co-authors, the life cycle of phytopathogenic fungi of the genus *Fusarium* and other plant pathogens demonstrates that the growth of these pathogens can be controlled with the antagonistic microorganisms at various phases of growth and development of the fungi [5].

But, despite the significant progress, there remains an unmet need for improved strains of microorganisms for their use in suppressing the causative agent of *Fusarium* wilt. Thus, there is a problem in finding new means of biological control of *Fusarium* wilt, which would allow to effectively prevent or inhibit the development and spread of the infection.

The purpose of this work was to isolate and screen strains of bacteria of the genera *Bacillus* and *Pseudomonas*, active against mycopathogens of the genus *Fusarium*, detected in seed material of winter wheat. **The objectives** of the study were:

- to isolate mycopathogens of the genus *Fusarium* from samples of affected seeds of winter wheat of Odeska semi-dwarf, Myronivska 808 and Odeska 51 varieties and to establish the frequency of their isolation;
- to study the biological properties and carry out species identification of the isolated *Fusarium* strains*;*
- to isolate bacterial strains of the genera *Bacillus* and *Pseudomonas* from natural sources, to establish the frequency of their isolation and to investigate their biological properties;
- to determine the antagonistic activity of isolated strains of *Bacillus* and *Pseudomonas* against mycopathogens of the genus *Fusarium* and to screen the most active antagonistic strains;
- to investigate the activity of secondary exometabolites of selected strains of *Bacillus spp.* and *Pseudomonas spp.* and determine their minimum inhibitory concentrations.

Research materials and methods

37 samples of seed material of varieties of winter soft wheat, characterized by different field resistance to fungi of the *Fusarium* genus, were used in experimental studies (Table 1).

Table 1 – Studied samples of winter soft wheat seeds

Wheat variety	Number of samples	Field resistance to *Fusarium* wilt according a 10-point scale
Odeska semi-dwarf	13	favorable (3 points)
Myronivska 808	12	intermidiate resistant (4 points)
Odeska 51	12	tolerant (6 points)

To determine infection, the selected seed samples were previously disinfected by immersing for 30 seconds in a 70° of ethyl alcohol followed by washing with sterile water, then placed in envelopes made of sterile filter paper for germination at a temperature of 25°C, air humidity of 70% according to DSTU 4138:2002 [2]. Seeds with visible signs of damage were selected for further research: isolation of pure cultures of phytopathogens, study of their biological properties, and species identification using traditional biological methods [15]. Biochemical properties were studied using API bioMerieux assays (BioMérieux, Marcy l'Étoile, France), according to the manufacturer's instructions [APIWEB™]. The

results were interpreted visually and decoded using a database APIWEB [APIWEB™].

The samples of soil taken from the fields of Odesa region, roots, seeds of infected and healthy plants, leaves' and plant' residues of winter wheat affected by *Fusarium* were used for the isolation of antagonistic active microorganisms.

30 samples of roots, seeds, leaves, and plant residues were taken from each source. The selected samples were treated with a 70% ethanol solution for 30 seconds, followed by washing with sterile water. After that, the samples were ground in a sterile mortar and 10-fold dilutions were prepared. 100 µl of suspensions at a dilution of 10^{-5} were applied on the surface of King B medium and MPA in Petri dishes and cultivated at a temperature of 28°C for 4 days.

The block method was used in the screening for antimycotic activity of isolated bacterial strains [16]. The strains of *Bacillus spp.* and *Pseudomonas spp.* were cultivated on MPA and King A media for 7 days for obtaining the crude extracts of secondary metabolites. The experiment was carried out in two variants to find out the effect of the composition of the media on the activity of the extracts:

1) the biomass of cultures grown in nutrient media was transferred to flasks with 100 cm^3 of liquid organic media: medium MPB – for *Bacillus spp.*, medium King B – for *Pseudomonas spp.*;

2) the manipulations were the same as in the first variant, but instead of organic media, the mineral media were used: medium Gause – for *Bacillus spp.*, medium Tar – for *Pseudomonas spp.*

The flasks were incubated at 28°C on a rotary shaker with constant stirring at 150 rpm for 72 h. The broth culture of the strains was filtered through sterile filter paper, and then the supernatant was centrifuged at 6000 rpm for 20 min and sterilized through Millipore 0.45 µm filters to remove bacterial cells. Cell-free supernatants were acidified to pH 2 with concentrated HCl to precipitate secondary metabolites, kept for 16 h at 4°C, and centrifuged at 10.000 rpm for 20 min. The precipitate was removed and extracted three times with 10 cm^3 of 100% methanol [17]. Methanolic crude extracts were evaporated on a rotary evaporator and redissolved in 1 cm^3 of pure methanol, then filtered through the 0.2 µm filter and stored at −80°C for further studies.

Antagonistic activity of the extracts against *Fusarium* was determined by the well method [18]. The obtained extracts were thawed, diluted with pure methanol to a concentration of 8 mg/cm^3 and 50 µl were introduced into the wells of the KA medium inoculated with *Fusarium* strains, and methanol was introduced into the control well. The evaluation of the results was carried out 10 days after incubation at 28°C, measuring the size of the growth inhibition zones of *Fusarium* strains.

To determine the minimum inhibitory concentration (MIC), that is, the concentration at which the absence of visible growth of the *Fusarium* strain was observed, the extracts, starting with a concentration of 8 mg/cm^3, were diluted 2 times (each subsequent one was 2 times smaller than the previous one). The methanolic extracts were used in this experiment in concentrations of 8 mg/cm^3, 4 mg/cm^3, 2 mg/cm^3 and 1 mg/cm^3.

The strains of phytopathogenic bacteria *Xanthomonas sp.* O/M15 and *Clavibacter sp.* T131, isolated from affected agricultural plants, were also used for a preliminary assumption of the chemical nature of the selected extracts.

Studies were performed 3 times. Statistical and graphical analysis of the results were performed using the Microsoft Office Exel-2016 program.

Results of the research and their discussion

One of the features of *Fusarium* wilt is participation in the pathological process of a complex of various species of the genus *Fusarium*. Quite often, representatives of 10-15 different species of the genus *Fusarium* can be isolated from one grain sample [19].

The infection with mycelial fungi of seed material of 3 varieties of winter soft wheat with different degrees of field resistance to *Fusarium* wilt was investigated at the first stage of the work. The mycopathogens were isolated in 75.7% of cases during the study of 37 seed infected samples (Fig. 1).

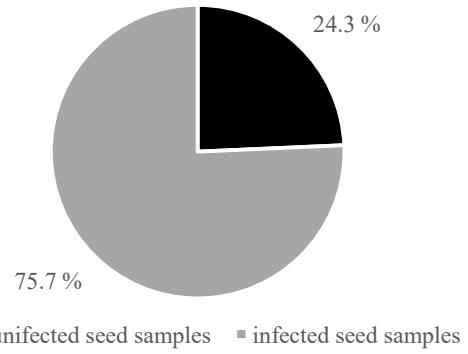


Fig. 1. The detection frequency (%) of damage by mycopathogens of the studied samples of winter wheat seeds

The frequency of the release of mycelial fungi was different and depended on the wheat variety (Fig. 2). Thus, all examined seed samples of the Odeska semi-dwarf variety, which is susceptible to *Fusarium* wilt (Table 1), were found to be infected with mycopathogens. According to the literature, Odesa semi-dwarf is a soft winter wheat variety, obtained by crossing Krasnodarsky dwarf 1 x Odesa 51 varieties with subsequent individual selection of the semi-dwarf genotype. It is known that the crops of this variety significantly suffer from the causative agents of *Fusarium* wilt [9,10].

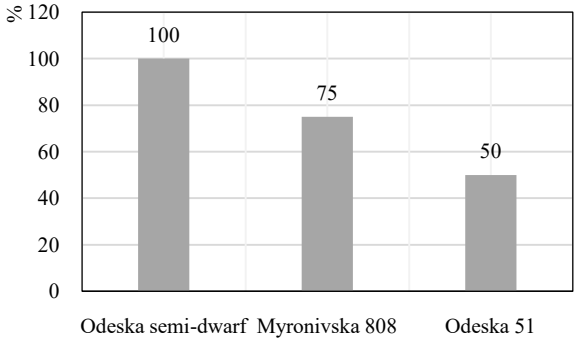


Fig. 2. The detection frequency (%) of damage by mycopathogens of seeds of different varieties of winter wheat

The seed material of the Odeska 51 variety, which is tolerant to the *Fusarium* causative agent, was the least affected by fungi: the frequency of the release of mycopathogens was 50.0% (Fig. 2). Odeska 51 is a high-quality perennial variety of winter soft wheat, that maintains resistance to *Fusarium* pathogens for a long time. All isolates were brought to the state of pure cultures after multiple passages on solid nutrient media Capek and KA.

Upon visual assessment of the colonies grown on nutrient media, it was found that they are white with a fluffy air structure. During microscopy, attention was drawn to conidiophores with micro- and macroconidia. These microstructures are typical of fungi of the genus *Fusarium.*

The following characteristics were taken into account for identifying micromycetes of the genus *Fusarium*: microscopic features (type and morphology of conidia; structure of conidial sporulation organs, presence or absence of chlamydospores); macroscopic features (structure and color of mycelium, colony growth, color of spore mass, presence or absence of sclerotia and their color).

Thus, based on the results of the study of the biological properties of micromycetes isolated from the seed material of winter wheat, three species of fungi of the genus *Fusarium* were identified: *F. proliferatum*, *F. oxysporum and F. graminearum*. The largest number of strains was represented by the species *F. graminearum* (Fig. 3).

F. graminearum strains cause significant damage to grain crops and they are particularly dangerous because their production the mycotoxin deoxynivalenol [20]. On the other hand, other species of the *Fusarium* genus (*F. oxysporum* and *F. proliferatum*, which were represented by 9 and 4 strains, respectively), are capable of producing quite dangerous toxins (fumonisins) in large quantities, which in plants lead to disruption of cell growth and cell differentiation, permeability of cells and apoptosis, and in animals they cause pronounced pathological changes, leading to damage to the glycocalyx and premature apoptosis [21].

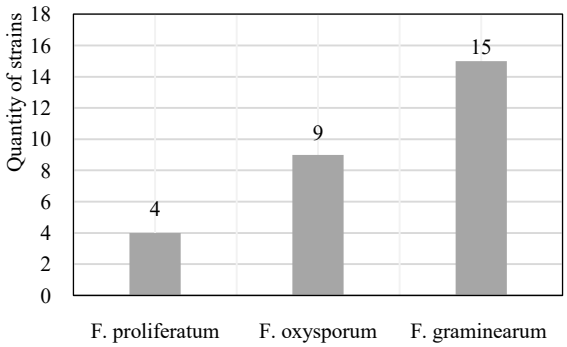


Fig. 3. The number of isolated strains of different species of the *Fusarium* genus

Considering the obtained results and the data of many publications, the issue of combating *Fusarium* wilt and its pathogens is quite relevant [20-22]. One of the promising and relatively safe directions in solving this problem is the creation of biological preparations based on biologically active strains of microorganisms. According to literature sources, representatives of the genera *Bacillus* and *Pseudomonas*, which produce various antimicrobial substances, are among the most promising in the defence of wheat from phytopathogenic mycomycetes [4,8,23].

Therefore, the next stage of the work was the isolation of *Bacillus spp.* and *Pseudomonas spp.* from various natural sources (soil samples, rhizosphere, roots, seeds and plant residues of winter wheat). 79 strains of *Bacillus spp.* and 34 strains of *Pseudomonas spp.* were isolated, brought to microbiological purity and identified as a result of the work carried out.

The largest number of strains was isolated from the rhizosphere zone of plants – 45 strains (36 and 9 strains *Bacillus spp.* and *Pseudomonas spp.*, respectively) (Fig. 4). In our opinion, this is due to the presence of a large amount of nutrients in an accessible form in this source. Slightly fewer strains of both genera were isolated from soil samples.

The prospects of strains of the genera *Bacillus* and *Pseudomonas* for use in plant protection are evaluated, first of all, by their antagonistic activity, that is, by the ability to inhibit or prevent the growth of pathogens of infectious diseases [24,25].

During the initial screening, all isolated strains *Bacillus spp.* and *Pseudomonas spp.* were tested for their ability to reveal antagonistic activity against isolated strains *Fusarium spp.* The vast majority of isolated strains of the genera *Bacillus* and *Pseudomonas* showed antagonism to at least one strain of *Fusarium spp*. 92.4 % of isolated strains *Bacillus spp.* and 73.5 % of isolated strains *Pseudomonas spp.* had antagonistic activity (Fig. 5).

The display of antagonistic activity of different bacilli strains against *F. graminearum* Se4 is demonstrated in Fig. 6.

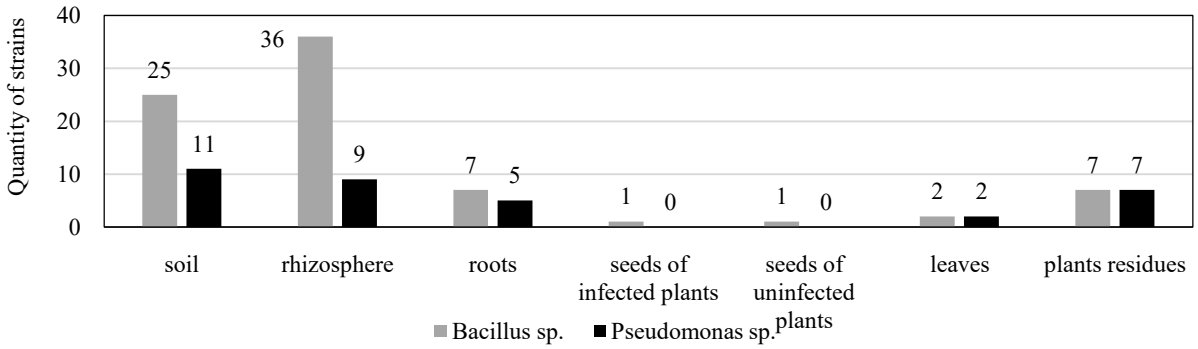


Fig. 4. Number of bacterial strains isolated from different natural sources

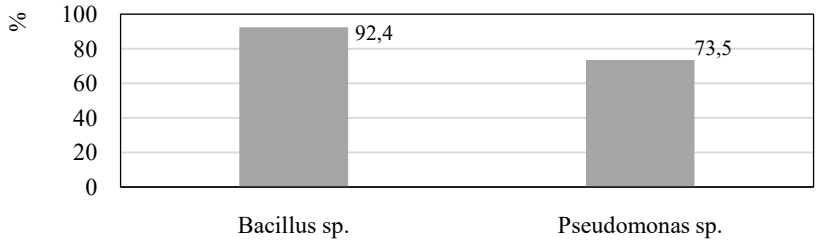
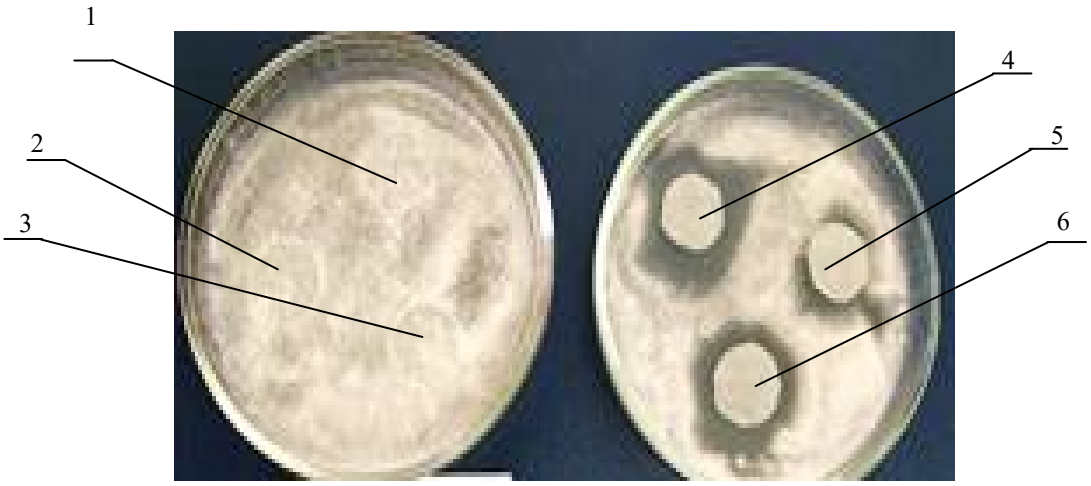


Fig. 5. The quotient (%) of isolated strains Bacillus spp. and Pseudomonas spp. that showed antagonistic activity against Fusarium spp.



Note: 1 – S1, 2 – Ro5, 3 – Ro6, 4 – S19, 5 – R10, 6 – R26

Fig. 6. Antagonistic activity of strains *Bacillus spp*. against *F. graminearum Se4*

When evaluating and analyzing the obtained results, certain regularities were confirmed, to which many researchers estimated in the relevant publications [23,24]. First, antagonistic activity depends on the source of the isolation of antagonistic microorganisms and is characteristic not only of specific species, but also of specific strains of these species (in particular, the degree of its manifestation). Secondly, the sensitivity of pathogens also depends on the strain that caused the disease. Thirdly, the display of activity depends on the conditions of its implementation, in particular the environment. In addition, this feature of antagonistic microorganisms' strains can weaken, so strains of pathogens can form mechanisms of resistance to antibiotic substances of producers. As a result of secondary screening, 11 strains *Bacillus spp.* and 12 strains *Pseudomonas spp.*, which showed antagonistic activity to all isolated mycomycetes strains, were selected. Tables 2 and 3 show the results of determining the size of growth inhibition zones of individual strains of various species of the *Fusarium* genus under the action of secondary metabolites of selected strains *Bacillus spp*. and *Pseudomonas spp*.

As can be seen from the obtained data presented in Tables 2 and 3, the sizes of the growth inhibition zones of *Fusarium spp*. were quite variable and ranged from 14.11±0.02 to 20.63±0.03 mm under the action of exometabolites of *Bacillus spp*. and from 11.27±0.02 to 16.82±0.02 mm under the action of exometabolites of *Pseudomonas spp.*

Table 2 – Growth inhibition zones of isolated *Fusarium spp*. strains (mm) under the action of the most antagonistically active *Bacillus spp*. Strains

Strains *Bacillus spp.*	*F. graminearum* Se4	*F. oxysporum* Se17	*F. proliferatum* Se28
R14	20.63±0.03	20.22±0.03	20.43±0.03
R27	19.81±0.02	19.21±0.03	19,12±0.02
R31	20.44±0,02	20.43±0.02	20.45±0.03
R33	19.41±0.01	19.42±0.03	19.57±0.03
R35	19.32±0.03	20.22±0.01	18.67±0.02
S4	18.84±0.02	18.53±0.02	19.77±0.03
S17	19.13±0.03	19.45±0.02	19.23±0.01
S19	20.42±0.03	20.56±0.03	20.45±0.02
Ro2	15.31±0.03	16.11±0.03	16.24±0.03
Ro3	15.21±0.02	15.33±0.02	16.00±0.03
Re1	14.11±0.02	15.09±0,02	15.23±0.03

Table 3 – Growth inhibition zones of isolated *Fusarium spp*. strains (mm) under the action of the most antagonistically active *Pseudomonas spp.* Strains

Strains *Pseudomonas spp.*	*F. graminearum* Se4	*F. oxysporum* Se11	*F. proliferatum* Se1
WR2	15.14±0.01	16.12±0.01	15.78±0.02
WR3	14.98±0.01	13.15±0.02	14.34±0.01
WR5	16.48±0.01	16.51±0.02	16.40±0.02
WR7	16.76±0.03	16.82±0.02	16.54±0.02
WR 8	15.12±0.02	15.44±0.02	15.65±0.01
WR9	14.28±0.01	14.89±0.02	13.76±0.03
WS2	15.02±0.01	16.21±0.02	15.34±0.02
WS4	14.78±0.02	14.64±0.02	15.10±0.02
WS11	13.98±0.02	14.55±0.03	14.87±0.,02
WRo1	14.27±0.03	14.45±0.02	14.84±0.,02
WRo4	11.27±0.02	12.24±0.02	11.67±0.02
WRo5	11.84±0.02	12.12±0.02	12.43±0.01

Among the selected bacilli, there were the most strains isolated from the rhizosphere (R14, R27, R31, R33, R35) and soil (S4, S17, S19) and they showed the best antagonistic activity against all isolated *Fusarium* strains.

For further work, strains of *Bacillus spp*. R14, R31 and S19, isolated from the rhizosphere and soil, were selected. Under the action of metabolites of these strains revealed the growth inhibition zones of all *Fusarium spp.* exceeded 20 mm, which indicates their considerable antagonistic potential.

On the other hand, under the action of even the most active antagonists among *Pseudomonas spp.*, the sizes of the growth inhibition zones of *Fusarium spp*. were smaller than under the action of the most antagonistically active strains *Bacillus spp*. Selected strains *Pseudomonas spp*., which inhibited the growth of all *Fusarium* species, were isolated from the rhizosphere, soil, and roots. Among the 12 studied *Pseudomonas spp.* strains, the strains WR5 and WR7, isolated from soil, were characterized by the highest antagonistic activity. Under the influence of the secondary metabolites of these strains, the size of growth inhibition zones of all *Fusarium* strains exceeded 16 mm.

Thus, as a result of sequential screening, 3 strains of *Bacillus spp.* R14, R31, S19 and 2 strains of *Pseudomonas spp.* WR5, WR7 were selected for further research.

The next stage was obtaining crude extracts of secondary metabolites of the selected bacterial strains and studying their activity against isolated *Fusarium spp*.

Taking into account the fact that one of the factors affecting the intensity of antagonistic activity is the composition of the culture medium, the antagonistic strains were cultivated on two types of media: organic (MPB – for *Bacillus spp.* and King B – for *Pseudomonas spp.*) and mineral (Gause 1 and Tar - for *Bacillus spp.* and *Pseudomonas spp.*, respectively) for obtaining of secondary metabolites extracts.

To study the biological activity of the obtained extracts, they were diluted with pure methanol to a concentration of 8 mg/cm^3 and 50 µl were introduced into the wells of the KA medium previously inoculated with *Fusarium* strains. In this study, one strain of each of the three species of Fusarium genus was used: *F. graminearum* Se4, *F. oxysporum* Se11, *F. proliferatum* Se1. The obtained results are shown in Table 4.

Table 4 - Antagonistic activity of methanol extracts of *Bacillus spp.* and *Pseudomonas spp.*

Extract (8 mg/cm^3)	Growth inhibition zones of *Fusarium* strains, mm		
	***F. graminearum* Se4**	***F. oxysporum* Se11**	***F. proliferatum* Se1**
***Bacillus sp.* R14 (MPB)**	17.46±0.02	17.46±0.02	18.23±0.03
***Bacillus sp.* R14 (Gause 1)**	8.02±0.03	10.11±0.02	10.22±0.03
***Bacillus sp.* R31 (MPB)**	15.28±0.02	16.34±0.02	18.41±0.02
***Bacillus sp.* R31 (Gause 1)**	7.80±0.02	8.42±0.01	7.39±0.02
***Bacillus sp.* S19 (MPB)**	18.45±0.02	18.68±0.02	18.54±0.01
***Bacillus sp.* S19 (Gause 1)**	11.28±0.02	10.44±0.02	11.67±0.02
***Pseudomonas sp.* WR5 (King B)**	15.78±0.02	15.78±0.02	15.95±0.01
***Pseudomonas sp.* WR5 (Tar)**	8.02±0.01	7.54±0.03	8.26±0.02
***Pseudomonas sp.* WR7 (King B)**	15.83±0.02	14.62±0.02	15.38±0.02
***Pseudomonas sp.* WR7 (Tar)**	8.23±0.02	7.81±0.03	7.75±0.02

The obtained results confirm the data of Bidima et al. [25] and Mona et al. [26], that the synthesis of secondary metabolites (both in terms of quantitative indicators and the diversity of their composition) depends on the component composition of the nutrient medium on which the producers are grown. In all cases, the studied strains *Bacillus spp*. and *Pseudomonas spp*. showed better antifungal activity when cultivated on organic media. That is, the compositions of the used organic media contribute to the accumulation of exometabolites by the producer strains. Despite the fact that the sizes of the growth inhibition zones of *Fusarium spp.* were somewhat smaller than under the influence of producer biomass (tables 2, 3), they were quite significant (from 15.28±0.02 to 18.68±0.02 mm under the action of exometabolites of *Bacillus spp*. and from 14.62±0.02 to 15.95±0.01 mm under the action of exometabolites of *Pseudomonas spp*.). On the other hand, the antagonistic activity of both *Bacillus spp*. and *Pseudomonas spp*. significantly decreased (approximately 1.5-2 times depending on the strain) when they were grown on appropriate mineral media. Taking into account the obtained data, 2 strains of bacilli (*Bacillus spp.* R14, S19) and *Pseudomonas sp.* WR5 were selected for the next stage of work, for the determining the minimum inhibitory concentrations (MIC) of their methanolic exometabolites.

Bacillus spp. R14, S19 and *Pseudomonas sp*. WR5 were grown, respectively, on MPA and King B, received methanol their extracts, after which they were diluted to concentrations of 8 mg/cm^3, 4 mg/cm^3, 2 mg/cm^3 and 1 mg/cm^3 and used in the experiment. In addition to the isolated *Fusarium* strains (*F. graminearum* Se4, *F. oxysporum* Se11, *F. proliferatum* Se1), 2 strains of phytopathogenic bacteria (*Xanthomonas sp.* O/M15, *Clavibacter sp.* T131) from affected agricultural plants were used in this experiment. The obtained results are shown in Table 5.

Methanolic extracts of *Bacillus spp.* R14, S19 and *Pseudomonas sp*. WR5 suppressed the growth of both phytopathogenic fungi and bacteria. At the same time, the MIC of extracts of *Bacillus sp*. R14 was 2 mg/cm^3 for *F. graminearum* Se4 and *F. oxysporum* Se11, and 1 mg/ml for *F. proliferatum* Se1.

Table 5 - Minimum inhibitory concentrations of methanolic extracts of *Bacillus spp*. and *Pseudomonas spp*. antagonistic strains

Extract	Concentration, mg/cm^3	*F. graminearum* Se4	*F. oxysporum* Se11	*F. proliferatum* Se1	*Xanthomonas sp.* O/M15	*Clavibacter sp.* T131
Bacillus sp. R14	8	+++	+++	+++	++	++
	4	++	+	+++	±	±
	2	±	±	+	-	-
	1	-	-	±	-	-
Bacillus sp. S19	8	+++	+++	+++	+++	++
	4	+++	+++	++	+	±
	2	++	±	±	±	-
	1	±	-	-	-	-
Pseudomonas sp. WR5	8	+++	+++	+++	+	++
	4	+	+	++	±	+
	2	±	±	±	-	±
	1	-	-	-	-	-

Note: «+++» - the size of the growth inhibition zone is more than 15 mm; "++" - the size of the growth inhibition zone from 10 mm to 14 mm; "+" - the size of the growth inhibition zone from 5 mm to 9 mm; "±" - the size of the growth inhibition zone is less than 4 mm; "-" - there is no growth inhibition zone

The MICs were higher and amounted to 4 mg/cm^3 for both strains of phytopathogenic bacteria (*Xanthomonas* sp. O/M15 and *Clavibacter sp.* T131). MIC of extracts of *Bacillus sp*. S19 was 2 mg/cm^3 for *F. oxysporum* Se11 and *F. proliferatum* Se1, 1 mg/cm^3 for *F. graminearum* Se4; concentrations of 4 mg/cm^3 and 2 mg/cm^3 were inhibitory for *Clavibacter sp*. T131 and *Xanthomonas sp.* O/M15, respectively. MICs of crude extracts of *Pseudomonas sp.* WR5 were 2 mg/cm^3 for all strains of mycopathogens and *Clavibacter sp*. T131, for the *Xanthomonas sp.* O/M15 this indicator was 2 times higher.

Analyzing the obtained results and comparing them with available literature data [25,26], it is possible to assert the presence of metabolites with both antimycotic and antibacterial activity in crude pools of extracts of *Bacillus spp*. and *Pseudomonas spp*. Moreover, it contains more antifungal substances. We can assume that polyketides and siderophores are among the secondary exometabolites of the studied bacilli strains, since bacilli are able to synthesize these substances with antifungal activity. Regarding the inhibition of the growth of representatives of the genera *Xanthomonas* and *Clavibacter*, it is possible that this is related to the synthesis of lipopeptides and siderophores, which can be produced by bacilli and are known to have antibacterial activity. The spectrum of *Pseudomonas spp.* exometabolites is quite large and diverse. Microbiologists distinguish siderophores, antibiotics, phenazines and their analogues, pigments, organic acids, ethers, etc. among the exometabolites of *Pseudomonas spp.* with antimicrobial activity. Therefore, to determine the spectrum and profiles of metabolites with antibiotic activity, it is necessary to conduct more comprehensive studies, including high-resolution mass spectrometry and mass spectrometry with metabolite imaging, as well as full sequencing and annotation of the genomes of *Bacillus spp*. R14, S19 and *Pseudomonas sp.* WR5, which will make it possible to identify gene clusters of secondary metabolites and carry out accurate species identification of strains.

Conclusion

The approach in the fight against *Fusarium* pathogens should be multi-component, starting from seed protection in the soil, to several treatments during the growing season [7]. The conducted research is a component of a complex approach, which consists in studying the species composition of phytopathogens of the genus *Fusarium*, searching for resistant wheat varieties, and searching for strains of antagonistic bacteria for the creation of biological preparations to reduce the *Fusarium* load. The infection rate of grain of various varieties of winter wheat, which differ in the degree of resistance to *Fusarium* wilt, was quite high and amounted to more than 75%. The study of biological properties made it possible to attribute the isolated strains to three species of the genus *Fusarium*: *F. proliferatum*, *F. oxysporum* і *F. graminearum*. The largest number of strains was represented by the species *F. graminearum*. 79 strains of *Bacillus spp.* and 34 strains of *Pseudomonas spp*. were isolated from various natural sources (soil samples, rhizosphere, roots, seeds and plant residues of winter wheat). The largest number of strains of both genera of bacteria was isolated from the rhizosphere zone of plants and soil. According to the results of sequential screening, 3 strains *Bacillus spp.* R14, R31, S19 and *Pseudomonas spp*. WR5 and WR7 were selected, which showed the highest antagonistic activity against all isolated *Fusarium spp*. Methanol extracts of secondary metabolites of *Bacillus spp*. and *Pseudomonas spp*. are 1.5-2 times more active than during the cultivation of producers on organic nutrient media. Minimum inhibitory concentrations of extracted exometabolites of *Bacillus spp.* R14, S19 are determined in the range from 1 mg/cm^3 to 4 mg/cm^3 depending on the strain of bacilli and the tested pathogen, for *Pseudomonas sp*. WR5 – from 2 mg/cm^3 to 4 mg/cm^3.

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ВІДБІР ШТАМІВ МІКРООРГАНІЗМІВ ДЛЯ ЗАХИСТУ ЗЕРНОВИХ РОСЛИН ВІД ГРИБІВ РОДУ *FUSARIUM*

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Анотація. У теперішній час через зміну клімату та низку несприятливих екологічних умов спостерігається тенденція до зниження світового виробництва пшениці - однієї з основних зернових культур. Незаперечно, що збільшення виробництва зерна щільно пов'язані з ефективністю контролю одних із найшкідливіших хвороб зернових культур – фузаріозів, оскільки абсолютно несприйнятливих до фузаріозу сортів пшениці у світі не існує. Розробка біотехнологічних підходів отримання нових мікробних препаратів для захисту озимої пшениці від збудників фузаріозу актуальна для біологічного захисту пшениці озимої в технологіях органічного землеробства і в системах інтегрованого захисту, істотно знижуючи ксенобіотичний прес на агроценози. Метою роботи було виділення і скринінг штамів бактерій родів *Bacillus* і *Pseudomonas*, активних проти мікопатогенів роду *Fusarium*, виявлених у насіннєвому матеріалі озимої пшениці. Інфікованість мікопатогенами роду *Fusarium* пщениці, районованої у південному регіоні, залежала від польової стійкості сорту та склала понад 75% випадків. Основним збудником фузаріозів були штами *F. graminearum*, також виділялися *F. oxysporum* та *F. proliferatum*. Із природних джерел було виділено 79 штамів бактерій роду *Bacillus* і 34 штами бактерій роду *Pseudomonas*. Найкращими антагоністами до усіх виділених фузарій були штами *Bacillus spp.* R14, R31 і S19 та *Pseudomonas spp.* WR5 і WR7. Метанолові екстракти вторинних екзометаболітів досліджених штамів бацил і псевдомонад проявили в 1,5 – 2 рази вищу активність проти фузарій за культивування продуцентів на органічних поживних середовищах. Мінімальні інгібуючі концентрації екстрагованих метаболітів штамів *Bacillus spp.* R14, S19 визначені в діапазоні 1 мг/см^3 – 4 мг/см^3, штаму *Pseudomonas sp.* WR5 – в діапазоні 2 мг/см^3– 4 мг/см^3.

Ключові слова: озима пшениця, мікопатогени роду *Fusarium*, бактерії-антагоністи, екзометаболіти.