INFLUÊNCIA DO TIPO DE SOLO E DA CULTURA ANTERIOR NA SUPRESSÃO DO FUSÁRIO POR BACTÉRIAS FORMADORAS DE ESPOROS LOCAIS

THE INFLUENCE OF SOIL TYPE AND PRECEDING CROP ON THE SUPPRESSION OF FUSARIUM BY INDIGENOUS SPORE-FORMING BACTERIA

ВЛИЯНИЕ ТИПА ПОЧВЫ И ПРЕДШЕСТВУЮЩЕЙ КУЛЬТУРЫ НА ПОДАВЛЕНИЕ FUSARIUM МЕСТНЫМИ СПОРООБРАЗУЮЩИМИ БАКТЕРИЯМИ

PRAZDNOVA, Evgeniya V. ^{1*}; GOROVTSOV, Andrey V. ^{2,3}; CHISTYAKOV, Vladimir A. ⁴; VASILCHENKO, Nikita G. ⁵; KUKHARENKO, Lidiya E. ⁶;

¹Southern Federal University, Academy of Biology and Biotechnology, Department of Genetics, 194/1 Stachki Ave., zip code 344090, Rostov-on-Don – Russian Federation

²Federal Rostov Agricultural Research Center, 1 Institutskaya Str., zip code 346735, Rassvet – Russian Federation

³Southern Federal University, Academy of Biology and Biotechnology, Department of Biochemistry and Microbiology, 194/1 Stachki Ave., zip code 344090, Rostov-on-Don – Russian Federation

⁴Southern Federal University, Academy of Biology and Biotechnology, Laboratory of Experimental Mutagenesis, 194/1 Stachki Ave., zip code 344090, Rostov-on-Don – Russian Federation

⁵Southern Federal University, Academy of Biology and Biotechnology, Department of Genetics, 194/1 Stachki Ave., zip code 344090, Rostov-on-Don – Russian Federation

⁶LLC Rostov Management Company, 152151, 18 Perovsky Lane, Rostov – Russian Federation

* Correspondence author

e-mail: ldsz@rambler.ru

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RESUMO

Restaurar e melhorar a fertilidade do solo, aumentar a produtividade das plantas cultivadas é uma das tarefas da produção agrícola. As bactérias do solo formadoras de esporos estão atraindo crescente interesse como agentes de biocontrole, mas a questão da influência das condições locais do solo no desenvolvimento de antagonismo em estirpes bacterianas locais não é bem conhecida. Portanto, o principal objetivo do trabalho é estudar a influência do tipo de solo e de culturas anteriores na intensidade das interações antagônicas entre as bactérias do solo da ordem *Bacillales* e os fungos fitopatogênicos. Para identificar as espécies e estirpes especificadas, os autores utilizaram o método de Sanger, a detecção de produtos de sequenciamento foi realizada automaticamente por eletroforese capilar. O critério para classificar um microrganismo como uma espécie específica foi considerado homologia de pelo menos 97%. Este estudo apresenta novas evidências sobre o efeito do tipo de solo e cultura anterior no nível de antagonismo em relação a *Fusarium* e *Plectosphaerella*. Como resultado, determinou-se que o tipo de solo e a cultura anterior influenciam as interações antagonistas fúngico-bacterianas. A atividade antagonista mais forte entre todos os isolados bacterianos foi encontrada em bactérias isoladas de *Albic Phaeozem*. A maior atividade antagônica contra *F. Graminearum* é representada por estirpes isoladas de solos nos quais o trigo de inverno era a cultura anterior.

Palavras-chave: *Bacillus*, *Paenibacillus*, *Fusarium*, biocontrole, antagonismo, tipo de solo, rotação de culturas.

ABSTRACT

Restoring and improving soil fertility, increasing the productivity of cultivated plants is one of the objectives of agricultural production. The aim of the present work was to study the antagonistic interactions between the soil bacteria of the order *Bacillales* and phytopathogenic fungi. The spore-forming soil bacteria attract increasing interest as biocontrol agents, but little is known about the influence of local soil conditions on

the development of antagonism in indigenous bacterial strains. This can lead to unsuccessful attempts of bacterial antagonists isolation. To determine the sequences of primary nucleotide DNA, the authors used the Sanger sequencing method, the detection of sequencing products was performed automatically, using the method of capillary electrophoresis. Homology of at least 97% was considered as the criterion for classifying a microorganism as a certain species. This study presents new data on the influence of soil type and preceding crop on the level of antagonism against *Fusarium* and *Plectosphaerella*. The results show that both the soil type and preceding crop influence the fungal-bacterial antagonistic interactions. The strongest antagonistic activity among all bacterial isolates was found in bacteria isolated from *AlbicPhaeozem*. The highest antagonistic activity against *F. graminearum* was shown by strains that were isolated from soils on which winter wheat was the preceding crop.

Keywords: *Bacillus*, *Paenibacillus*, *Fusarium*, biocontrol, antagonism, soil type, crop rotation.

АННОТАЦИЯ

Восстановление и повышение плодородия почвы, рост продуктивности культурных растений – одна из задач сельскохозяйственного производства. Спорообразующие почвенные бактерии привлекают все больший интерес в качестве агентов биоконтроля, но вопрос о влиянии местных почвенных условий на развитие антагонизма у местных штаммов бактерий недостаточно изучен. Поэтому основная цель работы заключается в изучении влияния типа почв и предшествующих культур на интенсивность антагонистических взаимодействий между почвенными бактериями отряда *Bacillales* и фитопатогенными грибами. Для идентификации выделенных видов и штаммов авторами был использован метод Сэнгера, детекцию продуктов секвенирования осуществляли автоматически методом капиллярного электрофореза. Критерием для отнесения микроорганизма к определенному виду считалась гомология не менее 97%. В данном исследовании представлены новые данные о влиянии типа почвы и предшествующей культуры на уровень антагонизма в отношении *Fusarium* и *Plectosphaerella*. В результате было определено, что тип почвы и предшествующая культура влияют на грибово-бактериальные антагонистические взаимодействия. Наиболее сильная антагонистическая активность среди всех бактериальных изолятов была обнаружена у бактерий, выделенных из *Albic Phaeozem*. Наибольшая антагонистическая активность против *F. Graminearum* представлена штаммами, которые выделялись из почв, на которых озимая пшеница являлась предшествующей культурой.

Ключевые слова: *Bacillus*, *Paenibacillus*, *Fusarium*, биоконтроль, антагонизм, тип почвы, севооборот.

1. INTRODUCTION

Due to the growing demand of the population for high-quality and safe agricultural products, there is a need to develop competent approaches and strategies for the cultivation of agricultural crops. The most important branch of agriculture is crop production.

The specialization of farming associated with repeated or permanent cultivation of agricultural plants leads to a number of problems. One of these problems is soil fatigue, which is caused by the depletion of the reserves of organic and mineral substances necessary for plant growth and development (Triberti *et al.*, 2016). In addition to the depletion of soil resources, this approach leads to the increased damage caused by various diseases. In grain crops, these include root rot, fusarium head blight, brown rust, scab and late blight in potatoes, cercosporiosis in sugar beet, and many other diseases of agricultural crops.

These diseases are caused by soil phytopathogenic microorganisms, remaining on crop residues and multiplying in the presence of suitable climatic conditions under permanent sowing (Bankina *et al.*, 2017; Benitez *et al.*, 2017).

Winter wheat is one of the main crops cultivated in Russia. That is why fusarium head blight has become widespread. The disease is observed in most regions where wheat is grown and causes serious losses up to 25-30% of the yield (Gagkayeva *et al.*, 2012; Gagkaeva, GavriloVA, 2014). The causative agents of this group of diseases are the fungi of the genus *Fusarium*. These fungi pose a serious threat to humans and farm animals due to the production of mycotoxins, namely fumonisin (Tamura *et al.*, 2015), zearalenone (Brodehl *et al.*, 2014), and the trichothecium series toxins: deoxynivalenol (DON), T-2-toxin and HT2-toxin. The toxins accumulate in the seeds of plants (Jimenez-Garcia *et al.*, 2018; Szabó *et al.*, 2016).

There are various ways to protect the crops from phytopathogenic fungi, with chemical fungicides being the most commonly used tool. However, in a number of works, it was shown that the effectiveness of the fungicides decreases steadily due to the gradual development of resistance to active substances (Becker R. *et al.*, 2010; Gasich *et al.*, 2015). At present, the vector of research has shifted from the search for chemicals to the search for bacteria and their metabolites, which show antagonistic properties to various fungal pathogens of agricultural crops (Parnell *et al.*, 2016). This is due to the demand for food free of agrochemical residues and the use of organic farming (Moreno-Velandia *et al.*, 2019).

One of the promising groups of microorganisms that differ both in their ability to effectively resist phytopathogens and to show phytostimulating properties is the aerobic spore-forming soil bacteria of the order Bacillales.

International experience indicates a high efficiency of using biological preparations based on bacteria of this order to control *Fusarium*. In particular, their effectiveness has been demonstrated in such crops as corn, cucumber, and potatoes (Cavaglieri *et al.*, 2005; Chen *et al.*, 2010; Sadfi *et al.*, 2001). The mechanism of action is the colonization of plant root during seed germination and the suppression of pathogenic fungi by excretion of chitinolytic enzymes, siderophores and specific antimicrobial peptides belonging to the fengycin, iturin and surfactin families. Aerobic spore-forming bacteria are frequently used in biopharmacology and biotechnology (Cochrane and Vederas, 2016). Their ability to form endospores allow them to be formulated with longer shelf-life due to higher resistance to stress conditions (Lazarovits *et al.*, 2014). Both biotic and abiotic factors affect the expression of genes related to antagonistic activity in *Bacillus* species (Díaz *et al.* 2013; Zapata and Díaz, 2012). This can lead to unsuccessful attempts of bacterial antagonists isolation from a number of environments.

It is known that soil bacteria are highly sensitive to local soil and climatic conditions – the type of soil, temperature, humidity, the composition of local microbial communities. Therefore, it is important to study the influence of these conditions on the incidence of antagonistic strains of soil bacteria. This approach allows increasing the chances to isolate a potent bacterial strain. At the same time, the strains of bacteria isolated from a specific region should be a perfect choice as a biocontrol agent for the

same region.

This paper is aimed to evaluate the influence of soil conditions and cultivated crops on the incidence and strength of antagonism in soil bacteria against *Fusarium* species.

2. MATERIALS AND METHODS

2.1. Soil sampling sites and methods

Soil samples were collected from agricultural fields located in the Labinsky and Kanevskoy districts of the Krasnodar region (southern Russia) (Figure 1). The sampling sites were located in steppe zone (1 and 2) or foothill zone (3-8). Soils were classified as Haplic Phaeozem, Albic Phaeozem, Luvic Chernozem, and Haplic Chernozem. Each marker represents approximate location of the sampling sites, as from two to seven separate fields were sampled at each location. The total number of fields that have been sampled was 28.

All but two fields were used for growing winter wheat, and the rest were used for growing winter barley. The preceding crops were soybean, corn, sugar beet, sunflower, winter wheat, and peas. The sampling sites location and corresponding soil types and crops are presented in Table 1.

Each field was sampled according to generally accepted methods (Gregorich and Carter, 2007). Ten subsamples of soil were collected from the 0-10 cm layer in the corners and the middle of each field. Then the subsamples were thoroughly mixed.

2.2. Isolation of fungal strains

The fungal strains were isolated from plant residues of wheat and corn, collected from fields 1, 5, 6 and 7. 10 g of plant residues were thoroughly grinded, then stirred with sterile water on a rotary shaker for 30 min. The resulting suspension was plated on wort agar supplemented with streptomycin and grown for 5 days at 25 °C. The colonies with morphology characteristic of *Fusarium* sp. were isolated, and grown on sucrose-water agar to enhance the production of macroconidia (Mansour *et al.*, 2012). Five strains of *Fusarium* sp. were isolated and used for further studies and species-level identification.

Strain Fus 1 was isolated from corn stem residues from site 1 (soil type – Haplic Chernozem), Poc 6.1 from site 6 (soil type – Luvic Chernozem), Poc 6.2.1 and Poc 6.2.2 from site 7 (soil type – Haplic Phaeozem), Ple1 from

site 5 (soil type – Albic Phaeozem).

2.3 Isolation of bacterial strains with antagonistic properties

The bacterial strains were isolated from the mixed soil samples. The soil was mixed with sterile water at a ratio 1:10 and ground with a rubber pestle to separate the bacterial cells from the soil particles. The resulting soil suspension was shaken in conical flasks at a rotary shaker for 30 min. To isolate spore-forming bacteria, the soil suspension was heated to 80 °C for 20 min. After cooling to room temperature, the dilutions were prepared and plated on the agar medium, that was composed of 50% wort agar and 50% nutrient agar.

At the same time, the pure culture of *Fusarium graminearum* (Fus 1) that had been grown on wort agar for 7 days prior to the experiment was washed with sterile 0,85% saline solution to obtain the suspension of conidia. The conidia in the resulting suspension were counted under the microscope using a hemocytometer, and the suspension was diluted to 10⁴ cells/ml. 50 µl of this suspension was plated on the same plates that were inoculated with soil suspension.

The colonies of bacterial strains were observed after 5 days of incubation at 30°C. The colonies with antagonistic properties were suppressing the growth of the fungus (Figure 2). These strains were isolated and transferred to the same medium for further study.

2.4 The measurement of antagonistic activity of the bacterial isolates

A total of 1040 strains of aerobic spore-forming bacteria have been isolated from the 28 mixed soil samples. The antagonistic activity of the isolates was measured by the method of inhibition zone diameter. The pure culture of each strain was subcultured on a fresh nutrient agar slant and incubated for 18 h to obtain a culture in the logarithmic growth phase. Then one loop of biomass was inoculated to the perimeter of an agar plate. Five strains of bacteria were tested on the same plate, and the test was performed in triplicate. The center of the plate was inoculated with the mycelium of *Fusarium graminearum*, and allowed to grow for 7 days.

The inhibition zone was measured as a perpendicular between the edge of the bacterial colony and the edge of the visible growth of the mycelium of the fungus.

The 202 strains exhibiting the highest

antagonistic activity (inhibition zone diameter >12 mm) was then tested against 4 additional fungal isolates to compare with their activity against *F. graminearum*.

2.5. The identification bacterial and fungal isolates

The genotypic characterization of the strains was based on sequence analysis of ribosomal genes. Conservative primers of genes encoding 18S rRNA were used for the primary identification of fungi (Innis *et al.*, 2012). To establish the phylogenetic relationship of related species, the method of comparing nucleotide sequences encoding the 5.8 S rRNA gene and the internal transcribed spacers ITS1 and ITS2 (Naumova *et al.*, 2007), as well as sequences encoding the D1 / D2 gene of the 26S rRNA, was also used.

Variable regions of genes encoding 16S rRNA were used to identify bacteria.

The criterion for classifying a microorganism to a particular species was considered to be homology of at least 97%.

DNA isolation. The isolation of DNA was performed with AmpliTube RV M-SorbTube kit (Syntol, Russia). The bacterial cells were grown on solid nutrient agar for 20 hours. The biomass was washed from the plates with a sterile saline solution and then centrifuged at 3000 g for 15 min. The pellet was frozen in liquid nitrogen and heated to 60 °C thrice. The quantity of DNA was estimated using Qubit 3.0 fluorimeter.

DNA purification was performed using the Genomic DNA Purification Kit (Syntol, Russia).

PCR reaction was performed with PCR Screen Mix Kit (Evrogen, Russia). The total volume of the PCR mixture was 25 µL. The concentration of primers was 0.5 mkM for all primer pairs (Table 1). Amplification was performed in thermal cycler T100 Thermal Cycler (Bio-Rad, USA). The protocol of amplification:

1. Preliminary denaturation at 95 °C – 3 min
2. 35 cycles of amplification as described above
3. Denaturation at 95 °C - 30 sec
3. Annealing of primers at the optimal temperature (Table 2) – 30 sec
4. Elongation at 72 °C – 30 sec
6. The last elongation at 72 °C – 5 min

Conditions for **electrophoresis** of PCR samples were: 1.0% agarose gel, 5 V/cm in TAE buffer for 30 minutes. If it was not possible to establish the species belonging only using

16SRNA, species-specific primers (Table 2) were used for accurate identification of some bacterial strains. Sequencing was performed on an AE3000 automatic sequencer. For the analysis of sequences, a specialized computer programs BLAST (Basic Local Alignment Search Tool), and RDB II (Ribosomal Database Project II) were used.

Sequencing. Determination of primary nucleotide DNA sequences was performed by Sanger method with gene-specific primers and Big dye terminator v3.1 Cycle Sequencing Kit with modification (Applied Biosystems, USA) in a volume of 10 µl.

The reading was carried out with both primers (forward and reverse). The reaction was carried out using the Veriti amplifier (Applied Biosystems, USA). Detection of sequencing products was carried out automatically by capillary electrophoresis on the device ABI PRISM 3730 (Applied Biosystems, USA). The results were manually checked for mis-called nucleotides in Sequencing Analysis v. 5.4 software (Applied Biosystems, USA).

3. RESULTS AND DISCUSSION:

3.1 Identification of strains

Strains of fungi. The primary screening of the GenBank database showed that the strains studied belong to the following systematic group: *Eukaryota*; *Fungi*; *Dikarya*; *Ascomycota*; *Pezizomycotina*; *Sordariomycetes*; *Hypocreomycetidae*; *Hypocreales*; *Nectriaceae*; *Fusarium*.

Strain Fus1. Phylogenetic kinship analysis conducted using strains of closely related microorganisms showed that the closest to the studied strain are *Fusariumculmorum*, *Fusariumgraminearum* (*Gibberellazeae*), *Fusariumasiaticum*. Due to the ambiguity of the molecular identification of the Fus 1 strain, its macro- and micromorphological studies were carried out when grown on potato-glucose agar (PDA) and diagnostic medium, water clove agar (CLA), ensuring efficient formation and uniformity of macroconidia (Leslie and Summerell, 2006). The characteristic morphology of macroconidia (Figure 4) with a conical apical cell and a sole basal indicates the strain belongs to the species *Fusariumgraminearum*. According to the results of the analysis of the nucleotide sequence that encodes part of the rRNA genes, as well as on the basis of morphological analysis, it was established that the strain under study is closest

to the species *Fusariumgraminearum* (99% homology).

Strains 6.2.1, Poc 6.2.2, Poc.6.1. Based on the 18S analysis, nucleotide sequences encoding the 5.8 S rRNA gene and internal transcribed ITS1 and ITS2 spacers, and analysis of the D1 / D2 domain, it was found that the strains studied are closest to *Fusariumoxysporum* (98% homology).

Strain Ple1. Based on a similar analysis of 18S, 5.8 S rRNA gene and internal transcribed ITS1 and ITS2 spacers, this strain was identified as *Plectosphaerellacucumerina* (formerly *Fusariumtabacinum*)(98% homology).

Bacterial strains. A phylogenetic comparative study carried out using typical strains of closely related bacteria and results of analysis of sequences of variable regions of genes encoding 16S rRNA showed that *Paenibacilluspeoriae* was the closest to the studied strains R 3.13(99%), R 4.5 (99%), O 1.27(99%). The strain R 4.24 was identified as *Paenibacillus jamilae* (99%), and the strain R 5.31 as *Paenibacillus polymyxa* (99%). Sequencing of strains R 4.6 and V 3.14 and primary screening of the GenBank and RDP-II database showed that the strains belong to the following systematic Bacteria groups; *Firmicutes*; *Bacillales*; *Bacillaceae*; *Bacillus*, and the homology with several species of the genus *Bacillus* was 99% for both strains. When carrying out PCR analysis of the studied strain using the species-specific primers Bamy-f and Bamy-r, a PCR fragments of 747 bp was obtained (Figure 5). That indicates that the strain can be attributed to the species *Bacillus amyloliquefaciens*, the homology with which was, according to database analysis, 98% for R 4.6 and 99% V 3.14.

1. PCR fragment obtained using primers Bval-f and Bval-r
2. PCR fragment obtained using primers secYsubF and secYsubR
3. PCR fragment obtained using primers secYlichF and secYlichR
4. PCR fragment obtained using primers Bamy-f and Bamy-r
5. PCR fragment obtained using primers Bmoj-f and Bmoj-r
6. PCR fragment obtained using primers Batr-f and Batr-r
7. PCR fragment obtained using primers Bson-f and Bson-r

8. DNA Marker 1kb DNA GeneRuler (10000, 8000, 6000, 5000, 4000, 3500, 3000, 2500, 2000, 1500, 1000, 750, 500, 250 bp, from bottom to top)

Amplification of a fragment of 747 bp using primers Bamy-f and Bamy-r indicates that the strain can be attributed to the species *Bacillus amyloliquefaciens* (Table 3).

3.2 Statistical analysis of the results on growth inhibition

The analysis of the fungal growth inhibition, caused by the most active strains of bacteria has shown, that the sensitivity to the antagonistic substances, produced by bacteria varied significantly in different fungal species. *F. oxysporum* was more resistant than *F. graminearum*. The growth inhibition zone for that species was 1.72 – 1.50 times smaller, depending on the strain. *P. cucumerina* demonstrated intermediate resistance level (Table 4).

To find out how the sensitivity of different fungal strains depends on the properties of individual bacterial strains, a correlation analysis was performed. If the potent bacterial strains, producing larger quantities of antifungal substances suppress all the fungi to a greater extent, that will result in a strong positive correlation between the data sets for different fungal species. The correlation matrix is presented in Table 5.

The results show that for the most of fungal species, the correlation is positive and statistically significant, but its strength is weak, assuming that there are additional factors determining the extent of inhibition of each fungus by a certain bacterial strain.

To determine whether the resistance to bacterial strains is species- or strain-specific, we performed cluster analysis of the available data. The results are presented in Figure 6.

As shown in Figure 6, three species of phytopathogenic fungi form separate clusters, based on their inhibition by 202 bacterial strains. At the same time, all three strains of *F. oxysporum* formed a single cluster, suggesting that the resistance to inhibition by bacterial strains is more species-specific than strain-specific.

Interestingly, within the *F. oxysporum* cluster, two strains were separated from the third one. Both of these strains (*F. oxysporum* 2 and 3) were isolated from Haplic Phaeozem while *F.*

oxysporum 1 was isolated from Luvic Chernozem. This led to the assumption that soil type could play a significant role in the formation of fungal-bacterial antagonistic interactions.

3.3 The influence of soil type on the antagonistic properties of bacterial strains

The largest number of bacterial strains was isolated from chernozems of various types (of all 1040 strains, 912 were isolated from soils belonging to chernozems). The strongest antagonistic activity was characteristic of bacteria isolated from Albic Phaeozem (on average the diameter of the suppression zone was 15.95 mm, which is 1.63 higher than that of Luvic Chernozem strains, and 2.79 times higher than strains of Haplic Chernozem) (Figure 7,8).

However, the influence of soil type had a different pattern for the selection of 202 most active bacterial strains. The dependence of the inhibition zone from the soil type was largely similar for *F. graminearum*, but it was completely different in other fungal strains (Figures 2,3). All three strains of *F. oxysporum* had average suppression zone below 15 mm, irrespective of the soil type. *P. cucumerina* was more sensitive to the bacterial antagonism, but the influence of the soil type was also insignificant.

3.4 The influence of preceding crop on the antagonistic properties of bacterial strains

The influence of the preceding crop on the antagonistic activity of bacteria was assessed since it is known that the preceding crop affects the development of root rot in cultivated crops (Peralta *et al.*, 2018). This determines the frequency of phytopathogenic fungi and antagonist bacteria interaction, and as a result, the further resistance of agroecosystems to plant pathogens.

It can be clearly noted that the greatest antagonistic activity against *F. graminearum* was shown by those strains that were isolated from soils on which winter wheat was the preceding crop (the diameter of the growth inhibition zone was significantly higher than that of strains isolated from soils with any different preceding crop). (Figure 9).

The strains, isolated from fields with different preceding crops demonstrated less pronounced antagonistic properties against *F. oxysporum* compared to *F. Graminearum* (Figure 10). The preceding crops that were associated with the highest level of antagonism against

fungus species, other than *F. graminearum* were corn and soybean. However, this difference was statistically significant only in *F. oxysporum* 3.

4. Discussion

The growth suppression of phytopathogenic fungi that have been studied in this paper depends on the antifungal activity of bacterial strains. The mechanisms, underlying this activity involve the secretion of various metabolites that interact with fungal hyphae and lead to their lysis.

The strains of bacteria with the highest antifungal activity tested in this research belong to the genera *Bacillus* and *Paenibacillus*. A number of metabolites with antifungal activity are described in the literature for these genera:

- 1) Enzymes of the chitinolytic complex, glucanases and other lytic enzymes that can destroy the cell wall of fungi.
- 2) Polyketides – bacillaene, difficidin, macrolactin.
- 3) Oligopeptides.
- 4) Lipopeptides.

Chitinases. Some species of the *Bacillus* genus are capable of secreting chitinases, so they can use chitin as a carbon source (Wang and Chang, 1997). The following enzymes of the chitinolytic complex of bacteria are known to degrade chitin: exochitinase (capable of cleaving dimeric units of the polymer chain from the non-reducing end), endochitinase (cleaving chitobiose to N-acetylglucosamine) and N-acetylglucosaminidase (Howard *et al.*, 2003).

However, many strains with fungicidal activity do not possess chitinolytic enzymes.

Polyketides. Polyketides are one of the largest groups of bacterial secondary metabolites. They are represented by ensembles of fatty acids synthesized by polyketide synthases (PKS), nonribosomal enzymes with a modular structure (Hertweck, 2009). Polyketides have a wide range of biological effects, including antifungal activity. Polyenes have the highest antifungal activity among them (Bookshelf, 2010). They bind to ergosterol in the membrane of fungal cells and, thus, weaken the membrane, causing the leakage of K⁺ and Na⁺ ions, which leads to cell death (Harunari *et al.*, 2017).

Non-ribosomally synthesized peptides, and lipopeptides are also secondary metabolites of fungi and bacteria (Mongkolthanaruk, 2012). The genera *Bacillus*

and *Paenibacillus* are capable of producing such metabolites as surfactins, fengicins, fusaricidins, polymyxins, lichenisine, iturins, locillomucin. The enzymes of the family of nonribosomal peptide synthases (NRPS) synthesizing them have a domain structure and different functional sites (domains) of the protein molecule participate in the synthesis of one peptide antimycotic (Walsh, 2016).

The resulting peptides are short oligomers 2-48 amino acids long. They often include atypical elements such as D-amino acids, methylated versions of standard amino acids, non-proteinogenic, hydroxylated and glycosylated residues. They can exist in the cell as a heterogeneous mixture of isoforms that differ in the length of the alpha chain. It was shown that these compounds are resistant to heating to 100 °C and the action of proteinase K (Zalila-Kolsi *et al.*, 2016), which is probably due to the presence of atypical amino acids and their stereoisomers in their structure. The ratio of NRP is often specific for different strains of the same species, and activity against different strains of fungi also varies.

The mechanism of action of these oligopeptides and lipopeptides is associated with a violation of the integrity of the membranes. In this study, we found, that the bacterial strains from the same soils had a significantly lower impact on *F. oxysporum* in comparison to *F. graminearum*. The sensitivity of different species and strains of fungi to the action of these lipopeptides often depends on the phospholipid composition, the presence of sphingomyelin and the sterol content in the membranes. However, the observed effect could have been influenced by the initial selection of bacterial strains using *F. graminearum* as a target. This selection could lead to variation in genetic response in presence of different fungal species.

The genes that provide the processes of non-ribosomal synthesis, although they are expressed quite independently, may be influenced by the same regulators. In particular, it is known that the strain *B. subtilis* 168 does not produce NRP due to mutations in the *sfp* and *DegQ* genes, with NRPS genes present in its genome (Tsuge *et al.*, 2005). There may also be epigenetic regulatory mechanisms that facilitate the adaptation of bacteria to a particular phytopathogen antagonist. Thus, it has been shown that the synthesis of fungicidal lipopeptides, at least iturins, and fengicins, is modulated by chemical signaling substances

secreted by the pathogenic fungus into the rhizosphere (Cawoy *et al.*, 2015).

The fact that the bacteria that were isolated from the fields where wheat had been growing in the previous cycle of crop rotation showed the greatest activity against *F. graminearum* leads to an assumption that genetic and epigenetic adaptation of the mechanisms of antifungal activity to these conditions took place. *F. graminearum* is mainly affecting small-grain cereal crops, though it can be found in several weed species (Suproniene *et al.*, 2019). At the same time, *F. oxysporum* is known to have a much broader host range (Dean *et al.*, 2012). Our results show that the level of antagonism against *F. oxysporum* did not significantly depend on the preceding crop, contrarily to what was found for *F. graminearum*. It is known, that within the *F. oxysporum* species there are so-called *formae speciales*— groups of lineages that are specialized to cause the disease in particular plant species (Kistler, 2001). The results suggest that the bacteria do not adapt to express antagonism against a particular *formae speciales*, but rather against the *F. oxysporum* species as a whole. This leads to similar patterns of antagonistic activity for different strains of *F. oxysporum*.

The cases of soils being suppressive to some soil-borne diseases are well-documented in the literature (Steinberger *et al.*, 2019). At the same time, the influence of soil type on the level of bacterial antagonism against the phytopathogenic fungi has not been widely reported. The mechanisms that may contribute to the extent of pathogens suppression may include direct parasitism of soil bacteria on pathogenic fungal species or indirect mechanisms like competition for iron or causing the systemic resistance in host plants (Adesina *et al.*, 2007; Han *et al.*, 2000).

There is some evidence that soils that are suppressive to *Fusarium* wilts are most frequently neutral or alkaline (Alabouvette *et al.*, 1996). However, our results have shown, that the most potent antagonistic bacteria were found in Albic Phaeozem, with pH levels varying from 5,5 to 6,0. This finding can be explained by the fact that in acidic soils the crops are more frequently affected by *Fusarium* wilt (Fang *et al.*, 2012). The lowest level of *F. graminearum* growth inhibition was found in Haplic Chernozem and can be attributed to the high pH (at average, 7,5) and high content of CaCO₃ characteristic to this soil type. These conditions intensify the competition for iron and are highly unfavorable for the phytopathogenic soil fungi (Steinberger *et al.*, 2019). To conclude,

the results suggest, that soil types that are suppressing *Fusarium* due to their physico-chemical properties are less likely to develop biological suppression from increased incidence of potent antagonistic bacterial strains. This is probably due to more frequent competition between the bacteria and fungi in disease-conductive soil types. The lack of crop rotation further increase the chances of bacteria and fungi to meet in the rhizosphere of the host plant.

4. CONCLUSIONS:

Thus, it was established that soil type could influence fungal-bacterial antagonistic interactions. The strongest antagonistic activity among all bacterial isolates was found in bacteria isolated from Albic Phaeozem. However, this effect does not manifest for all selected strains of phytopathogens. The strongest dependence was found in antifungal activity against *F. graminearum*. The influence of the preceding crop on the antagonistic activity of bacteria was also determined. The greatest antagonistic activity against *F. graminearum* was shown by strains that were isolated from soils on which winter wheat was the preceding crop.

The most effective antagonists that were identified belonged to the genera *Bacillus* and *Paenibacillus*. These bacterial genera are known for a wide variety of nonribosomally synthesized metabolites, the production of which can be regulated by external factors. We could hypothesize that, during the crop rotation, the microbial part of the biocenosis adapts to specific phytopathogens and adjusts its metabolism to the most effective fight against them. All of these factors should be considered when selecting agents for the biocontrol of fungal diseases of crops.

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Table 1. Primers used for sequencing

Gene	Primer sequence
Fungi	
18S rRNA	NS1 -GTAGTCATATGCTTGTCTC NS4 – CTTCCGTCAATTCCTTTAAG
ITS1 and ITS2 spacers	ITS1 – TCCGTAGGTGAACCTGCG ITS4 – TCCTCCGCTTATTGATATGC
D1/D2 gene of 26S rRNA	NL-1 GCATATCAATAAGCGGAGGAAAG NL-4 GGTCCGTGTTTCAAGACGG
Bacteria	
16S RNA	8F – AGA GTTTGA TCC TGG CTC AG 926R – CCG TCA ATT CCT TTR AGT TT 1492R – GGT TAC CCT TGT TAC GAC TT

Table 2. Primers used for PCR identification

Species	Primers	Tm. °C	Amplicon size, bp
<i>Bacillus subtilis</i>	secYsubF TTATATCACGGCTTCGAT secYsubR CGGTAGTTTCGTTTCACCA	57	1050
<i>Bacillus amyloliquefaciens</i>	Bamy-f AAATCTGCCCCGATCGTCGGT Bamy-r GTGAGCATTGGCGTCACGGCG	60	747
<i>Bacillus licheniformis</i>	secYlichF TTACATCACAGCTTCTAT secYlichR CGATAGTTTCGTTTACCA	57	1050
<i>Bacillus atrophaeus</i>	Batr-f CACCCTCACGGAGATTCCGCA Batr-rAATTCTTTCTTTCCCTGATGG	54	541
<i>Bacillus mojavensis</i>	Bmoj-f CGTTATCGTATCCCGGGCA Bmoj-r AAAATTCTTTCTTTCCCTGAC	60	685
<i>Bacillus vallismortis</i>	Bval-f CGGATGTTCTGTGACGGTTTAC Bval-rCCGCAGTCGGGAAGTCAGGA	60	538
<i>Bacillus sonorensis</i>	Bson-f CTTGTTCAAGGCCATGGG Bson-r CCAAATGATGTTTGAAGT	57	684

Table 3. The results of identification of fungi and bacteria

Strain	Genbank accession number(s)	Species, according to rRNA gene analysis	% of homology
Fus 1	MN083273 (28 S) MN089640 (5,8 S /ITS1, ITS2)	<i>Fusarium graminearum</i>	99
Poc 6.2.1	MN219593 (18 S) MN219583 (5.8 S) MN219634 (D1/D2 26 S)	<i>Fusarium oxysporum</i>	98
Poc 6.2.2	MN219651 (18 S) MN219656 (5.8 S) MN219658 (D1/D2 26 S)	<i>Fusarium oxysporum</i>	98
Poc 6.1	MN103524.1 (18 S) MN219565 (D1/D2 26 S)	<i>Fusarium oxysporum</i>	98
Ple1	MN396230 (18 S) MN396231 (5.8 S)	<i>Plectosphaerella cucumerina</i>	98
R 3.13	MN220147 (16 S)	<i>Paenibacillus peoriae</i>	99
R 4.5	MN220138 (16 S)	<i>Paenibacillus peoriae</i>	99
R 4.6	MN220139 (16 S)	<i>Bacillus amyloliquefaciens</i>	97
R 4.24	MN220140 (16 S)	<i>Paenibacillus jamilae</i>	99
R 5.31	MN220144 (16 S)	<i>Paenibacillus polymyxa</i>	99
R 6.14	MN220145 (16 S)	<i>Paenibacillus peoriae</i>	99
V 3.14	MN220146 (16 S)	<i>Bacillus amyloliquefaciens.</i>	99
O 1.27	MN219728 (16 S)	<i>Paenibacillus peoriae</i>	98
K 1.14	MN219707 (16 S)	<i>Paenibacillus jamilae</i>	99.
O 2.11	MN220137 (16 S)	<i>Paenibacillus peoriae</i>	99

Table 4. The average growth inhibition zone diameters

Species	Inhibition zone diameter
<i>F. graminearum</i>	22,441±0,810
<i>F. oxysporum</i> 1	12,995±0,632
<i>F. oxysporum</i> 2	14,946±0,570
<i>F. oxysporum</i> 3	14,559±0,536
<i>P. cucumerina</i>	19,941±0,878

Data presented as mean±0,95 confidence intervals

Table 5. The correlations between the diameters of the growth inhibition zones for different fungal strains

Fungal species	1	2	3	4	5
1 <i>F. graminearum</i>	1.000				
2 <i>F. oxysporum</i> 1	0.237*	1.000			
3 <i>F. oxysporum</i> 2	0.218*	0.350*	1.000		
4 <i>F. oxysporum</i> 3	0.381*	0.281*	0.368*	1.000	
5 <i>P. cucumerina</i>	0.106	0.217*	0.296*	0.321*	1.000

* – correlation is significant at $p < 0,05$

Figure 1. The locations of the soil sampling sites

Figure 2. The primary isolation of strains with antagonistic properties

Figure 3. The measurment of the inhibition zone diameters

Figure 4. The characteristic morphology of macroconidia of *Fus1* strain on diagnostic medium.

Figure 5. PCR analysis of strain R 4.6 using species-specific primers

Figure 6. The results of cluster analysis based on growth inhibition zone diameters

Figure 7. The growth inhibition of *F. graminearum* caused by the bacterial isolates from different soil types

Figure 8. The growth inhibition of fungal strains caused by the bacterial isolates from different soil types

Figure 9. The growth inhibition of *F. graminearum* caused by the bacterial isolates from fields with different preceding crops

Figure 10. The growth inhibition of fungal strains caused by the bacterial isolates from fields with different preceding crops