Incidence of microorganisms antagonistic to plant pathogenic fungi *Bipolaris sorokiniana* and *Fusarium* sp. in different soil communities

E P Puchkova and V K Ivchenko

Krasnoyarsk State Agrarian University, 90 Mira Avenue, 660049, Krasnoyarsk, Russia

E-mail: puchkova_el@mail.ru

**Abstract.** The antibiotic activity of conidia germination of the fungi *Bipolaris sorokiniana* and *Fusarium* sp. in the culture filtrate of the antagonistic microorganisms identified in different soil communities have been quantitatively assessed. According to the research results, among 54 tested soil bacteria isolates, 26 have manifested a verified antagonistic activity to *Bipolaris sorokiniana*, 27 – to *Fusarium* sp. (TIG6), 36 – to *Fusarium* sp. (Y7ec), 40 – to *Fusarium* sp. (T2ec), as proven by the results of the mathematical treatment of the collected data (statistical significance p<0.01). It should be noted that the greatest number of the antagonists to plant pathogenic fungi *Bipolaris sorokiniana* were found in the soil collected from underneath the wheat of the Tulunskaya-12 breed. The maximum number of the organisms antagonistic to fungi *Fusarium* sp. were found in the soil taken from underneath indoor plants and sod-meadow soil. Preliminary identification of the antagonistic soil bacteria has shown that the majority of the antagonistic bacteria represent the coryneform bacteria, including *Arthrobacter* sp. and *Micobacterium* sp., *Bacillus* sp. and *Pseudomonas* sp., Actinobacteria group. A cluster and factor analysis of the test cultures by their sensitivity to the culture filtrate of the soil bacteria showed that the greatest similarity in the reaction to the bacterial metabolites is demonstrated by the isolates of *Fusarium* sp. T2ec and Y7ec. Compared to all isolates of *Fusarium* sp., *Bipolaris sorokiniana* show a significantly different reaction to the metabolites produced by soil bacteria. The x2 criterion-based statistic analysis did not show any difference in the incidence of the antagonistic microorganisms in the used test cultures.

1. **Introduction**

Plant diseases are known to be wide-spread and significantly harmful to agriculture. The chemical method is the most common one used to tackle the problem for a number of positive properties: quick and obvious effect, reliability and efficiency in a wide range of the external factors of the medium. However, in the last several years the negative effects of the method have become visible and tangible. Apart from the harmful microorganisms, chemical pesticides kill the beneficial microbiota, therefore destructing the soil ecosystems and breaking the soil fertility reproduction processes [1-3]. To create alternative methods of protecting the plants from diseases, the bacteria, actinomycetes and fungi capable of serving as agents for the biological disease control are being actively sought for. The biologicals based on the microorganisms antagonistic to the plant disease agents have a narrow spectrum, hardly affecting the soil microorganism communities, therefore maintaining the biological balance [4-10]. For
this reason, some antagonistic microorganisms were selected from different soil communities identified as the most active towards the plant pathogenic fungi Bipolaris sorokiniana and Fusarium sp.

The objective of this paper was to study the incidence of the microorganisms antagonistic to plant pathogenic fungi Bipolaris sorokiniana and Fusarium sp. in different soil communities

2. Methods and results
As an object of the research, different microbial soil communities were used: soil from underneath the Tulunskaya-12 wheat breed (Emelyanovsky District), garden soil (Emelyanovsky District), sod-meadow soil (Vetluzhanka Microdistrict) and mixed soil from underneath indoor plants. For the research, the plant pathogenic fungi Bipolaris sorokiniana and three strains of Fusarium sp. were selected and united into a pure culture of infected grain crop seeds.

The primary screening of the soil microorganisms was carried out with the co-culture method in the PD agar medium (fermentation peptone – 9.0, fermentation casein hydrolysate – 8.0, yeast extract – 3.0, sodium chloride – 5.0, sodium hydrogen orthophosphate – 2.0+0.5 pH (7.2+0.2)) and in Czapek medium ((g/l of solution) sucrose – 30, NaNO3 – 3; KH2PO4 – 1; MgSO4 * 7H2O – 0.5; KCl – 0.5; FeSO4* 7H2) – 0.01; agar – 15-20). For this purpose, one gram of soil was weighed and placed into a sterile flask with 100 ml of distilled water and thoroughly shaken. Then from the flask, 1 ml of the soil suspension was transferred to a Petri plate onto the surface of the agar medium previously seeded with Bipolaris sorokiniana and Fusarium sp. fungi conidia. The conidia were seeded with a microbiological spreader in three Petri plates one after another. The fungi were cultivated in a germination chamber at a temperature of +26°C. In the cultivation process, the bacterial colonies avoided by the plant pathogenic fungi were noticed (figure 1).

![Figure 1. Seeding and cultivation of soil microorganisms in the PD agar medium (photo by E P Puchkova).](image)
The quantitative assessment of the antibiotic activity was based on the germination of the *Bipolaris sorokiniana* and *Fusarium* sp. conidia in the culture filtrate of the identified antagonistic microorganisms. For this purpose, humid media, i.e. Petri plates with circles of humidified two-layer paper filters were used. On the first microscope glass, a drop of sterile water was applied (as a reference). On the second microscope glass, a drop of the culture filtrate of the first microorganism strain was placed. On the third microscope glass, a drop of the culture filtrate of the second microorganism strain was put, etc. The microscope glasses with the drops were placed in the humid media in the Petri plates. After that, into every drop, the *Bipolaris sorokiniana* and *Fusarium* sp. conidia were introduced [11]. The Petri plates were put in the germination chamber under the temperature of 26°C. Four hours later, the germinated conidia of *Bipolaris sorokiniana*, and seven hours later, those of *Fusarium* sp. were counted in the reference and the culture filtrate of the studied strains (figure 2, figure 3).

**Figure 2.** Inhibition of *Fusarium* sp. conidia germination in the culture filtrate of the identified antagonistic microorganisms (1) compared to the reference (2), x10 lens (photo by E P Puchkova).

**Figure 3.** Inhibition of *Bipolaris sorokiniana* conidia germination in the culture filtrate of the identified antagonistic microorganisms (1) compared to the reference (2), x10 lens (photo by E P Puchkova).
The microscopic survey was carried out with the Micmed 6 microscope equipped with a digital camera DCM-130E.

The mathematical treatment of the survey results was based on the Student's criterion. Moreover, the analysis of the arbitrarily sized contingency tables was carried out with the $\chi^2$ criterion. To integrate the test cultures based on their sensitivity to the soil bacteria culture filtrate, a cluster and factor analysis was used [12]. MS Office XP and StatSoft STATISTICA software were used.

The study of the impact made by the culture filtrate of the identified antagonistic bacteria to the germination of the fungi *Fusarium* sp. (strains Т2ес, TIG6, Y7ec) and *Bipolaris sorokiniana* showed that among 54 tested soil bacteria isolates, 26 manifest verified antagonistic activity to *Bipolaris sorokiniana*, 27 are antagonistic to *Fusarium* sp. (TIG6), 36 – to *Fusarium* sp. (Y7ec), and 40 – to *Fusarium* sp. (T2ec), as confirmed by the statistically processed data.

Preliminary identification of the antagonistic soil bacteria has shown that the majority of the antagonistic bacteria represent the coryneform bacteria, including *Arthrobacter* sp. and *Micobacterium* sp., *Bacillus* sp. and *Pseudomonas* sp., Actinobacteria group. The greatest number of the antagonists active against the fungi *Bipolaris sorokiniana* were found in the soil collected from underneath the wheat of the Tulunskaia-12 breed. The maximum number of the organisms antagonistic to fungi *Fusarium* sp. were found in the soil taken from underneath indoor plants and in sod-meadow soil (figure 4). The differences between the antagonistic activities of the strains are confirmed with the mathematical analysis results at the significance level of $p<0.01$.

![Figure 4](image)

*Figure 4.* Incidence of the antagonistic microorganisms in the studied soil communities.

A cluster and factor analysis of the test cultures by their sensitivity to the culture filtrate of the soil bacteria showed that the greatest similarity in the reaction to the bacterial metabolites is demonstrated by the isolates of *Fusarium* sp. T2ec and Y7ec. Compared to all isolates of *Fusarium* sp., *Bipolaris sorokiniana* show a significantly different reaction to the metabolites produced by soil bacteria (figure 5).
Figure 5. Test cultures grouped by sensitivity to the culture filtrate of the antagonistic microorganisms identified in the soil, based on the cluster (1) and factor (2) analyses.

Nevertheless, the χ² criterion-based statistic analysis did not reveal any statistically relevant differences between the studied soils in the general incidence of the microorganisms antagonistic to the used test cultures.

3. Conclusion
The greatest number of the antagonists active against the fungi Bipolaris sorokiniana were found in the soil collected from underneath the wheat of the Tulunskaya-12 breed. The maximum number of the organisms antagonistic to fungi Fusarium sp. were found in the soil taken from underneath indoor plants and sod-meadow soil. Some similarity of reaction to the metabolites of the antagonistic microorganisms was shown by the isolates of Fusarium sp. (T2ec and Y7ec). Compared to all isolates of Fusarium sp., Bipolaris sorokiniana showed a significantly different reaction to the metabolites produced by soil
bacteria. In the studied soil communities, no difference in the general incidence of the microorganisms, antagonistic to the plant pathogenic fungi *Bipolaris sorokiniana* and *Fusarium* sp. (strains T2ec, TIG6, Y7ec) was found. Therefore, all the soil communities studied in the present paper can be used to identify the organisms antagonistic to *Bipolaris sorokiniana* and *Fusarium* sp.

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