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ANTIFUNGAL ACTIVITY OF BACTERIAL SPECIES *Pseudomonas*

AKTYWNOŚĆ PRZECIWGRZYBOWA BAKTERII Z RODZAJU *Pseudomonas*

Abstract: The aim of the research was to determine a fungistatic activity of bacteria *Pseudomonas* against *Fusarium* sp. The antagonistic properties were assessed with a culture-plate method on Czapek and PDA growth media for *Pseudomonas* sp. cultures after 4, 6, 8, 10 and 24 hours of culturing. The culturing process was conducted at 22°C for 14 days. The fungistatic activity of *Pseudomonas* sp. strains was determined against the growth rate index. Obtained results proved that tested strain of *Fusarium* sp. was sensitive to supernatants of *Pseudomonas* spp. The linear growth of the mycelium of *Fusarium* sp. was inhibited most actively by *Pseudomonas* sp. strain BK1. The highest decrease, amounting 77%, in the value of the growth rate index was obtained after 10 hours of culturing on Czapek medium. The highest reduction of the growth rate index for *Pseudomonas* sp. strain KF1 was also noted for 10-hour culture, but it amounted only 42% in this case. Conducted research confirm fungistatic activity of *Pseudomonas* spp. strains against *Fusarium* sp. and prove that growth inhibition of the mycelium depends not only on the type of metabolites produced by a specific bacterial strain but also on time they were cultured for.

Keywords: fungistatic activity, growth rate index, *Pseudomonas* sp., *Fusarium* sp.

There has been a general concern about the amount of chemical substances applied in the process of plants protection. Therefore natural biological preparations containing non-pathogenic microorganisms have become more and more popular due to their high efficiency which allows to use them in place of fungicides. Investigations into properties of microorganisms in order to find the most suitable bacterial strains brought the attention to the activity of bacteria *Pseudomonas*.

Many non-pathogenic species of bacteria *Pseudomonas* belong to PGPR group (ie *Plant Growth Promoting Rhizobacteria*) and have an advantageous influence on plants by producing biologically active metabolites and growth regulating substances. Moreover, some of *Pseudomonas* spp. strains developed different mechanisms which limit pathogenic activity of fungi. According to literature sources [1, 2], bacteria possess the ability to synthesize different substances which are biologically active, ie hydrogen cyanide, siderophore, salicylic acid and lytic enzymes, proteases.

The aim of conducted research was to determine how metabolites produced by strains of *Pseudomonas* sp. KF1 and BK1 affect the growth of *Fusarium* sp.

Materials and methods

For the experiment, test strains of *Pseudomonas* sp. marked KF1 and BK1 isolated from the natural environment were used together with an indicator strain *Fusarium* sp.

Fungistatic activity of tested strains was determined with the culture-plate method on Czapek medium consisting of [g/dm³]: sucrose 30.0, MgSO₄ × 7H₂O 0.5, KH₂PO₄ 1.0, KCl 0.5, NaNO₃ 3.0, Fe₂(SO₄)₃ × 7H₂O 0.01, agar 15.0 and on PDA medium consisting of [g/dm³]: glucose 20.0, potato extract 4.0, agar 15.0. In bacterial cultures which were control

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trials, *Pseudomonas* sp. was added to the media as supernatants obtained after 4, 6, 8, 10 and 24 hours of culturing. Next, the media were inoculated with fungi discs of 10 mm diameter. The control trial contained *Fusarium* sp. cultures and aseptic broth medium in place of the supernatant. All plates were cultured at 22°C for 14 days. After 4-2 days the diameters were measured until *Fusarium* sp. on the plate with the control trial grew over the whole surface of the plate. The experiment was conducted in three trials, where one trial was represented by one plate with the growth medium and one mycelial disc.

The influence of metabolites produced by *Pseudomonas* sp. on the growth of *Fusarium* sp. was determined against the growth rate index, calculated according to the formula below [3]:

$$T = \frac{A}{D} + \frac{b_1}{d_1} + \frac{b_2}{d_2} + \dots + \frac{b_x}{d_x}$$

where: T - growth rate index, A - mean value of diameter measurements, D - the length of the experiment [number of days], b_1 , b_2 , b_x - increase in a diameter size since the last measurement, d_1 , d_2 , d_x - number of days since the last measurement.

Results

In the experiment, 2 bacterial strains *Pseudomonas* (KF1 and BK1) were tested against their ability to synthesize exocellular metabolites possessing fungistatic abilities in relation to *Fusarium* sp. On the basis of obtained results, it has been observed that fungistatic activity of tested *Pseudomonas* is varied and depends on the type of the growth medium and the length of culturing.

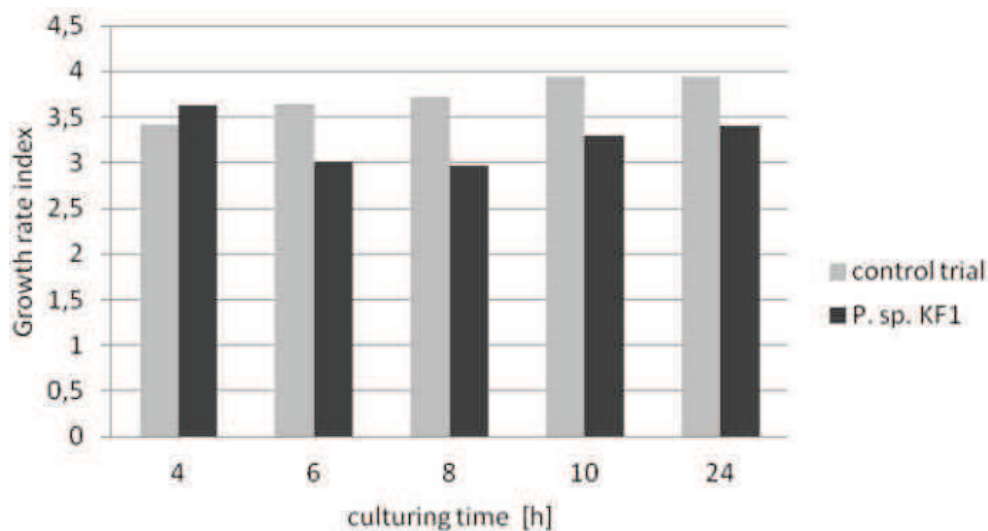


Fig. 1. The influence of *Pseudomonas* sp. KF1 on mycelial growth of *Fusarium* sp. on Czapek medium

The tests showed that strain *Pseudomonas* KF1 was least active in terms of its fungistatic activity on Czapek medium (Fig. 1). For the supernatant obtained after 4 hours of culturing, a decrease in the growth rate index has not been recorded in comparison with the control trial. Only after supplementing the growth medium with 6, 8, 10 and 24-hour cultures, the drop of the growth rate index was noticeable and reduction amounted between 14 and 20%. Linear growth of the mycelium was inhibited most actively by adding 8-hour culture to the growth medium.

The analysis of *Fusarium* sp. growth on PDA medium with an addition of supernatants of *Pseudomonas* sp. KF1 revealed much more intensive drop of the growth rate index than on Czapek medium (Fig. 2). The highest value of the growth rate index was noted for 8 and 10-hour cultures and obtained reduction amounted almost 40 and 42%. The lowest reduction in the value of the growth rate index was obtained in case of 4-hour culture supernatant.

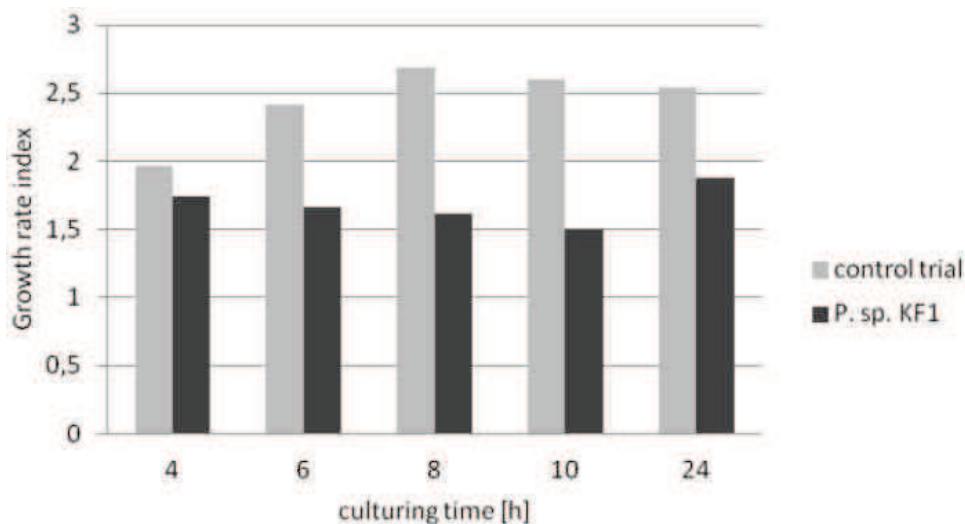


Fig. 2. The influence of *Pseudomonas* sp. KF1 on mycelial growth of *Fusarium* sp. on PDA medium

In presented tests, fungistatic activity in relation to *Fusarium* sp. was also determined for strain *Pseudomonas* sp. BK1. Recorded values of the growth rate index allowed to state that strain *Fusarium* sp. showed the fastest linear growth (Fig. 3) on Czapek medium. Only after 8 days from the beginning of the experiment, plates with control trials were grown over with mycelium, which covered its whole surface and the growth rate index for the consecutive trials fluctuated between 4.121 and 4.735. At the same time, the test trials were characterised by much lower value of the growth rate index, which resulted in the highest reduction of the mycelial growth rate. The reduction value was recorded as follows: 77% for the supernatant obtained from 10-hour culture and 70% for 24-hour culture. For the remaining cultures, the reduction in the growth rate index did not exceed 60%.

In case of *Pseudomonas* sp. BK1 cultured on PDA medium, a linear growth of the mycelium of *Fusarium* sp. was inhibited most actively when the supernatant obtained from

4-hour culture was added (Fig. 4). The reduction in the growth rate index amounted almost 60% when compared with the control trial. For the remaining trials, a lower reduction in the growth rate was recorded: from 25% for 24-hour culture to ca. 44% for 8-hour culture.

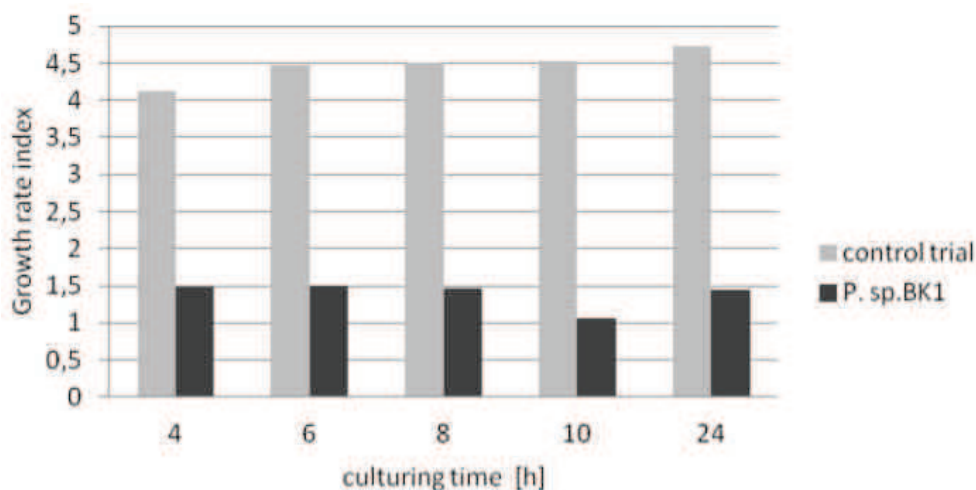


Fig. 3. The influence of *Pseudomonas* sp. BK1 on mycelial growth of *Fusarium* sp. on Czapek medium

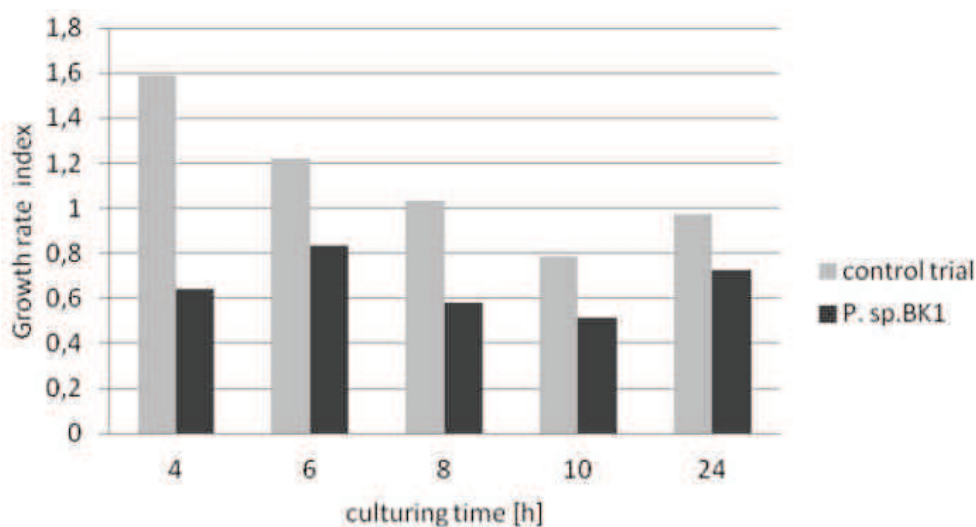


Fig. 4. The influence of *Pseudomonas* sp. BK1 on mycelial growth of *Fusarium* sp. on PDA medium

It is worth noticing that obtained values of the growth rate index for the control trials on Czapek medium were several times higher in comparison with the ones obtained on PDA medium for the both *Pseudomonas* sp. KF1 and *Pseudomonas* sp. BK1 strains. It may probably result from different sources of carbon in the growth medium (glucose or sucrose)

and different preference towards them showed by tested strain of *Fusarium* sp. Moreover, for most of the trials the linear growth of the mycelium of *Fusarium* sp. was increasing with the time of culturing.

Summary and conclusions

Conducted research showed significant diversity of fungistatic activity among *Pseudomonas* sp. strains. The growth of *Fusarium* sp. was inhibited most actively by the exocellular metabolites produced by *Pseudomonas* sp. BK1. The most optimal results have been obtained on Czapek medium where sucrose is a source of carbon. The lowest fungistatic activity was observed for *Pseudomonas* sp. KF1 and the growth medium for which the results were satisfactory was PDA medium with glucose. Judging from recorded results, an addition of the supernatants obtained from 8 and 10-hour cultures may significantly inhibit linear growth of *Fusarium* sp.

Strains of *Pseudomonas* sp. may be applied to protect plants against diseases caused by *Fusarium* sp. However, the difference in fungistatic activity between tested strains revealed in the experiment may be the evidence that it is typical for a specific strain, which depends not only on the time of culturing, but also on the type of the growth medium or the environment it is designed for.

References

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AKTYWNOŚĆ PRZECIWGRZYBOWA BAKTERII Z RODZAJU *Pseudomonas*

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Abstrakt: Celem badań było określenie aktywności fungistatycznej bakterii z rodzaju *Pseudomonas* wobec *Fusarium* sp. Ocenę właściwości antagonistycznych metabolitów przeprowadzono metodą hodowlano-płytkową na podłożu Czapka oraz PDA dla 4-, 6-, 8-, 10- i 24-godzinnych hodowli *Pseudomonas* sp. Hodowle prowadzono w temperaturze 22°C przez 14 dni. Na podstawie indeksu tempa wzrostu określono aktywność fungistatyczną szczepów *Pseudomonas* sp. Wyniki doświadczenia wskazują, że badany szczep *Fusarium* sp. był wrażliwy na działanie supernatantów *Pseudomonas* sp. Największą inhibicję rozrostu liniowego grzybni *Fusarium* sp. powodował szczep *Pseudomonas* sp. BK1. W tym przypadku najwyższy 77% spadek indeksu tempa wzrostu grzybni uzyskano w przypadku 10-godzinnej hodowli na podłożu Czapka. Natomiast dla szczepu *Pseudomonas* sp. KF1 największą redukcję indeksu tempa wzrostu odnotowano dla hodowli 10-godzinnej i wynosiła ona jedynie 42%. Przeprowadzone badania potwierdzają fungistatyczne działanie szczepów *Pseudomonas* sp. wobec *Fusarium* sp. Ponadto wykazują, że inhibicja wzrostu grzybni uzależniona jest nie tylko od rodzaju metabolitów wydzielanych przez dany szczep bakterii, ale również od wieku jej hodowli.

Słowa kluczowe: aktywność fungistatyczna, indeks tempa wzrostu, *Pseudomonas* sp., *Fusarium* sp.