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ANTIFUNGAL ACTIVITY OF BACTERIAL SPECIES *Pseudomonas* AGAINST *Alternaria* sp.

AKTYWNOŚĆ PRZECIWGRZYBOWA BAKTERII Z RODZAJU *Pseudomonas* WOBEC *Alternaria* sp.

Abstract: The aim of the research was to determine a fungistatic activity of bacteria *Pseudomonas* against *Alternaria* sp. The antagonistic properties of metabolites 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. As proved by the results obtained, the strain of *Alternaria* sp. under study showed sensitivity to supernatants of *Pseudomonas* sp. The linear growth of the mycelium of *Alternaria* sp. was inhibited most actively by *Pseudomonas* sp. strain BK1. The highest decrease, amounting 76%, in the value of the growth rate index was obtained after 10 hours of culturing on Czapek medium. In case of *Pseudomonas* sp. strain marked KF1, the highest reduction in the growth rate index was noted for the 24-hour culture on PDA medium and amounted to only 18%. Conducted research confirms fungistatic activity of *Pseudomonas* sp. strains against *Alternaria* sp. and proves that the growth inhibition of mycelium depends not only on the metabolites types produced by a specific bacterial strain but also on the length of culturing process.

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

Plant diseases caused by fungi such as *Alternaria* and *Fusarium* constitute the most numerous and the most significant group of diseases taking into account an economic aspect. In order to control them the fungicides are applied, which despite their efficiency and simplicity are not highly ranked in the process. First of all, the fungicides may be hazardous to consumers as their particles may remain on the plants. Moreover, the substances induce the development of pathogens' immunity to such chemicals and a reduction of harmless organisms. In addition, it is difficult and expensive to develop new and safe products. Therefore, there has been more and more interest in biological methods of plants protection [1]. Consequently, more popular have become biological substances based on non-pathogenic microorganisms, such as bacteria of *Pseudomonas* kind, which are of great interest to scientists looking for efficient strains to be used in the process. The special attention has been brought to: *P. fluorescens*, *P. aeruginosa*, *P. putida* and *P. pyrrocinia*. The strains show antifungal activity with varying degrees of antagonism. According to literature sources [2-4], 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 *Alternaria* sp.

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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 *Alternaria* 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 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 *Alternaria* sp. cultures and aseptic broth medium in place of the supernatant. All plates were cultured at 22°C for 14 days. After 2-4 days the diameters were measured until *Alternaria* 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 containing the growth medium with one mycelial disc.

The influence of metabolites produced by *Pseudomonas* sp. on the growth of *Alternaria* sp. was determined against the growth rate index, calculated according to the formula below [5]:

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

where: T - growth rate index, A - mean value of diameter measurements [cm], 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 *Alternaria* sp. On the basis of obtained results, it has been proved that fungistatic activity of tested *Pseudomonas* is varied and depends on the type of the growth medium and the length of culturing.

It has been observed, in conducted tests, that strain *Pseudomonas* KF1 was least active in terms of its fungistatic activity on PDA medium (Fig. 1). For the supernatants obtained after 6 and 8 hours of culturing, a decrease in the growth rate index has not been recorded in comparison with the control trial. As measured, the increase in the growth rate index amounted 3.48 and 7.15% respectively for the above. Only after having supplemented the growth medium with 4, 10 and 24-hour cultures, the drop of the growth rate index was noticeable and the reduction amounted between 2.72 and 18.17%. The linear growth of the mycelium was inhibited most actively when the supernatant of 24-hour culture was added to the growth medium.

The analysis of *Alternaria* sp. growth on Czapek medium with an addition of supernatants of *Pseudomonas* sp. KF1 revealed much more intensive drop of the growth rate index than on PDA (Fig. 2). The degree of obtained reduction, however, has not been high and amounted from 7.27% for the 6-hour cultures to 13.58% for the 24-hour culture.

In the control trials of *Alternaria* sp. the value of the growth rate index was raising with the length of culturing time and amounted 3.41 for 4-hour culture to obtain the value of 3.91 for 24-hour culture. Similar relationship has not been noted for *Alternaria* sp. growing on the medium with sucrose (*ie* Czapek medium).

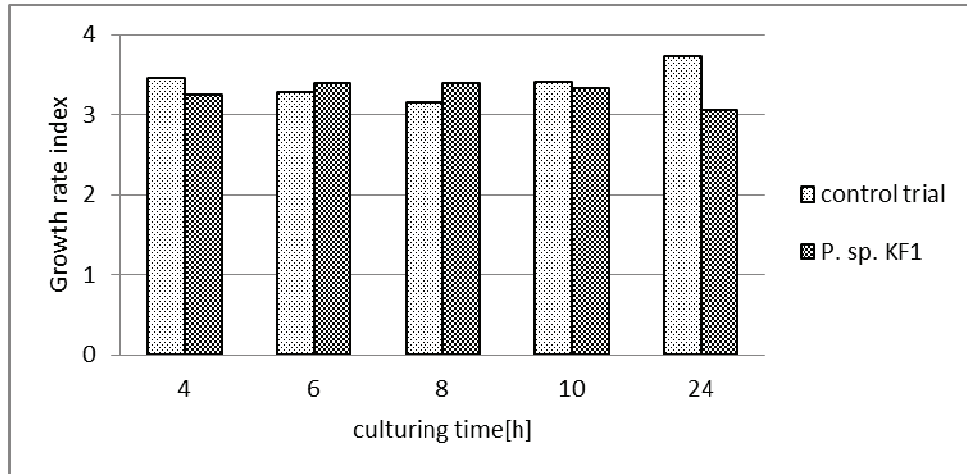


Fig. 1. The influence of *Pseudomonas* sp. KF1 on mycelial growth of *Alternaria* sp. on PDA medium

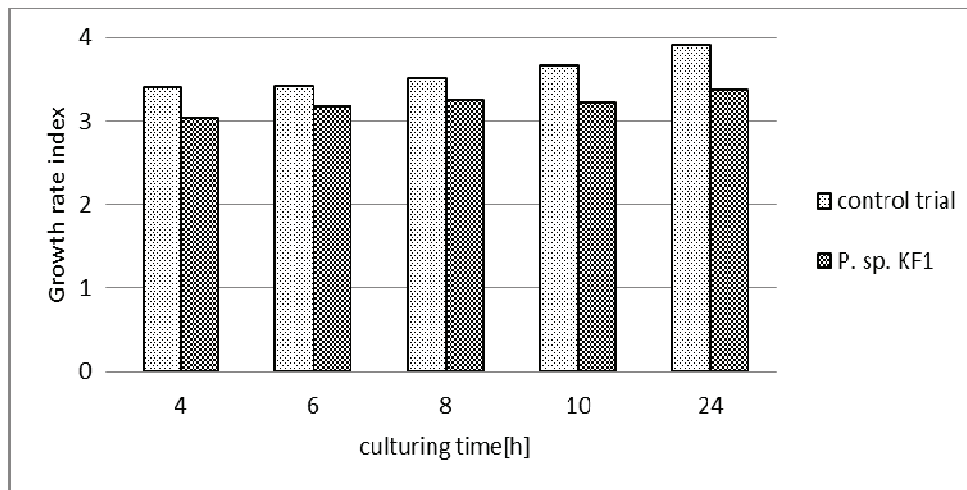


Fig. 2. The influence of *Pseudomonas* sp. KF1 on mycelial growth of *Alternaria* sp. on Czapek medium

In presented tests, fungistatic activity was also determined for strain *Pseudomonas* sp. BK1 against *Alternaria* sp. Consequently it has been revealed that supernatants obtained from 4-hour cultures caused significant inhibition of the linear growth of *Alternaria* sp. (Fig. 3) and measured value of the growth rate index reduction amounted 61.37% when

compared with the control trial. Similar reduction has been recorded for 10-hour culture (61.92%). Both results were the highest recorded values for such bacteria-fungus combination. For the remaining trials, a lower reduction in the growth rate was recorded: 59% for 6-hour culture and 57% for 8-hour culture. An antagonistic activity of *Pseudomonas* sp. BK1 was least efficient when the supernatant was obtained after 24 hours of culturing. The value of the growth rate index was the lowest for both the control trial (2.25) and the particular trial (1.54) and the decrease of index amounted only 31.5%.

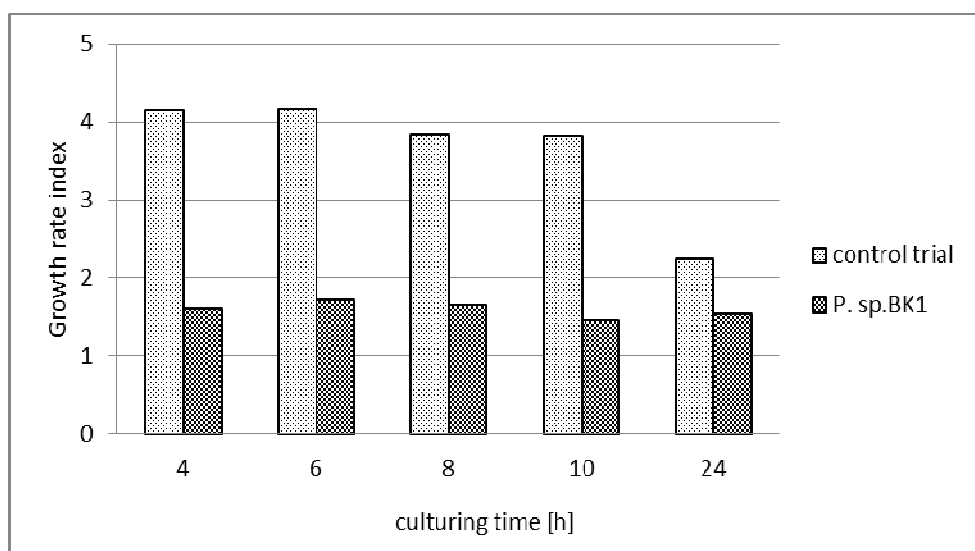


Fig. 3. The influence of *Pseudomonas* sp. BK1 on mycelial growth of *Alternaria* sp. on PDA medium

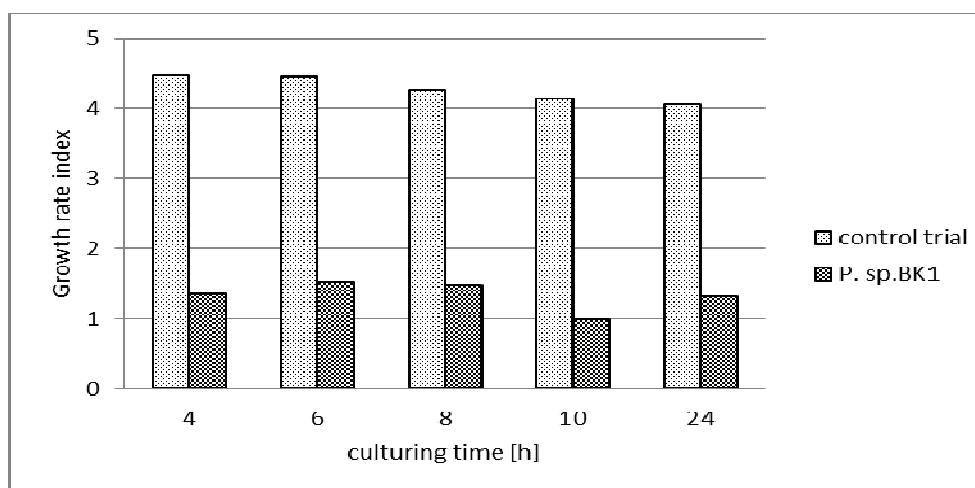


Fig. 4. The influence of *Pseudomonas* sp. BK1 on mycelial growth of *Alternaria* sp. on Czapek medium

On the basis of obtained results concerning the growth rate index it may be stated that strain *Alternaria* sp. showed the fastest linear growth on the medium with sucrose (Czapek medium) as showed in Figure 4. The plate was fully covered with the fungus only after 8 days of the experiment. The values of the growth rate index obtained in control trials were the highest throughout the whole experiment and fluctuated between 4.46 for 4-hour culture and 4.05 for 24-hour culture. At the same time, the value of the growth rate index in control trials was significantly lower and resulted in the highest reduction of the mycelial growth rate. It amounted to 76% in case of the supernatant obtained from 10-hour culture. The values obtained for other cultures were also high (65÷70%).

It is worth noticing that obtained values of the growth rate index for the control trials on Czapek medium were higher in comparison with the ones obtained on PDA medium. Therefore, in most cases, linear growth of mycelium was inhibited more actively on Czapek medium as noted for strains of *Pseudomonas* sp. KF1 and *Pseudomonas* sp. BK1. 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 *Alternaria* sp.

Summary and conclusions

Conducted research showed significant diversity of fungistatic activity among particular *Pseudomonas* sp. strains. The growth of *Alternaria* sp. was inhibited most actively by the exocellular metabolites produced by *Pseudomonas* sp. BK1. The most optimal results have been obtained on Czapek medium with sucrose as carbon source. The lowest fungistatic activity was observed for *Pseudomonas* sp. KF1 and the growth medium for which the results were satisfactory was the growth medium with sucrose. However, the growth inhibition was so low when compared with the results obtained for *Pseudomonas* sp. BK1, that it seems unlikely to apply the strain to control *Alternaria* fungi.

Moreover, on the basis of obtained results, it shows that the most favourable is to apply 10-hour cultures to inhibit the linear growth of *Alternaria* sp.

To sum up, strains of *Pseudomonas* sp. may find a wide range of applications in the process of plant protection against diseases caused by *Alternaria* sp. However, the differences 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 length of culturing time, but also on the type of the growth medium or the environment it is designed for.

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AKTYWNOŚĆ PRZECIWGRZYBOWA BAKTERII Z RODZAJU *Pseudomonas* WOBEC *Alternaria* sp.

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Abstrakt: Celem badań było określenie aktywności fungistatycznej bakterii z rodzaju *Pseudomonas* wobec *Alternaria* 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. Hodowlę 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 *Alternaria* sp. był wrażliwy na działanie supernatantów *Pseudomonas* sp. Największą inhibicję rozrostu liniowego grzybni *Alternaria* sp. powodował szczep *Pseudomonas* sp. BK1. W tym przypadku najwyższy 76% spadek indeksu tempa wzrostu grzybni uzyskano dla 10-godzinnej hodowli na podłożu Czapka. Natomiast dla szczepu *Pseudomonas* sp. KF1 największą redukcję indeksu tempa wzrostu odnotowano dla 24-godzinnej hodowli na podłożu PDA, jednak wynosiła ona jedynie 18%. Przeprowadzone badania potwierdzają fungistatyczne działanie szczepów *Pseudomonas* sp. wobec *Alternaria* 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., *Alternaria* sp.