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## ANTIFUNGAL ACTIVITY OF BACTERIAL SPECIES *Bacillus* AGAINST *Alternaria* sp.

### AKTYWNOŚĆ PRZECIWRZYBOWA BAKTERII Z RODZAJU *Bacillus* WOBEC *Alternaria* sp.

**Abstract:** The aim of the study was to assess the fungistatic activity of cell-free supernatants (CFS) obtained from 4, 6, 8, 10 and 24-hour culture of *Bacillus* KF2 and *Bacillus* BK2 against *Alternaria* sp. The antagonistic activity of bacterial exometabolites was determined by the dual-culture technique both on Czapek and PDA media. Plates were incubated at 28°C for 13 days and the fungal growth was measured every 2 days. The antagonistic activity of these strains was evaluated as the growth rate index. The growth of *Alternaria* sp. was 4-fold and 5-fold slower after application of CFS obtained from *Bacillus* KF2 (respectively on PDA and Czapek media) than in control. In case of 10-hour culture and 4-hour culture maximum antifungal activity of this strain was observed on PDA and Czapek media, respectively. This strain reduced the growth rate of *Alternaria* sp. from 72 to 80%. However, inhibition of *Alternaria* sp. by *Bacillus* BK2 was markedly less than that by *Bacillus* KF2. An increase in age of culture of *Bacillus* BK2 correspondent to an increase in the percent of inhibition up to 24-hour culture from 16% to 22% on PDA and Czapek media, respectively. Supernatants obtained from the culture of both strains showed fungistatic activity against *Alternaria* sp., depending on the age of the culture and the composition of the medium, although the *Bacillus* KF2 showed a stronger effect than *Bacillus* BK2. The experimental results exhibit the fungistatic activity of *Bacillus* strains and indicate the possibility of using theirs, especially *Bacillus* KF2, as antifungal agents against *Alternaria* sp.

**Keywords:** *Bacillus* sp., *Alternaria* sp., antifungal activity, growth rate index

### Introduction

Plant pathogens are the most important factors that cause major losses and damages to agricultural products every year. Phytopathogenic fungi affecting crop and post-harvested vegetables are major threat to food production and food storage. Moreover fungi are potential health hazard for humans due to the production by them toxic metabolites. Current agriculture is based largely on the application of synthetic pesticides and fungicides. The exercise use of agrochemicals lead to the emergence of pathogen resistance and serve negative impacts on the environment, cause serious effect to human health and non-target organisms. Therefore, it is a growing demand for new and safer methods to reduce, replace or at least supplement the existing control strategies. One of the most promising means to achieve this is the use of new tools based on *biological agents* (BCAs) for disease control [1-5].

In recent years, there has been a growing interest in potential use as plant protection non-pathogenic microorganisms isolated from natural environment as promising alternatives to synthetic chemicals. While diverse bacteria may contribute to the biological control of plant pathogens, most of the research and development efforts focused on two genera, *Bacillus* and *Pseudomonas*. *Bacillus* species posses several advantages that make them good candidates for use as BCAs. First, they produce several different (various) types

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of antimicrobial compounds, such as antibiotics, cell wall degrading enzymes and siderophores. Second, they induce growth and defence responses in the host. Furthermore, *Bacillus* sp. are able to produce spores that are resistant to UV light and desiccation, and that allow them to resist adverse environmental conditions and permit easy formulation for commercial purposes [1, 2, 5-7].

## Materials and methods

The involvement of antifungal compounds produced by the *Bacillus* strains KF2 and BK2 in the inhibition of fungal growth was confirmed by the ability of *cell-free culture* (CFS) filtrate of these strains to inhibit of hyphal growth of *Alternaria* sp. The *Bacillus* strains were inoculated into a flask containing the nutrient broth (OD (Optical density) = 2.0 at  $\lambda = 560$  nm) and incubated at 30°C for various times to give 4, 6, 8, 10 and 24-hour culture (working culture). The *Alternaria* sp. strain was cultivated on Czapek-Dox medium at 25°C for 5 days. The antagonistic activity of the tested *Bacillus* strains was evaluated as the rate index of fungal growth.

**Determination of influence of *Bacillus* strains on mycelium growth.** Fungal mycelial-disks (diameter of 10 mm) obtained from growing cultures of tested fungal isolates were placed in the centre of Czapek-Dox and PDA media that containing 0.5 cm<sup>3</sup> working cultures of *Bacillus* strains (in four replications). A control was made only with fungal mycelial-disks on both media without bacteria. After incubation at 27°C for 14 days, plates were observed at 2 days intervals and estimated as the rate *index of fungal growth* (*I<sub>fg</sub>*) using the formula [8]:

$$I_{fg} = \frac{A}{D} + \frac{b_1}{d_1} + \dots + \frac{b_x}{d_x}$$

where *A* is the mean from colony measurement, *D* is the experiment duration (days), *b<sub>1</sub>...b<sub>x</sub>* is the increase a colony diameter from lasted measurement, *d<sub>1</sub>...d<sub>x</sub>* is the number days from lasted measurement.

## Results and discussion

The antifungal activity of *Bacillus* KF2 and *Bacillus* BK2 strains grown on 2 different media was evaluated towards tested *Alternaria* sp. as the growth rate index. The results of the study showed that *Alternaria* sp. was sensitive to the metabolites produced by the tested strains from the genus *Bacillus* isolated from the natural environment. Fungistatic activity was determined by many factors, and depended mainly on the biological properties of these strains and their culture age, the growth media used and the susceptibility of the fungus. This might be due to secretion of metabolites secreted by these strains, diffused and dissolved into the culture media.

Promising results have been achieved particularly for *Bacillus* KF2. This strain was the most active on the Czapek medium, where the carbon source was sucrose (Fig. 1).

An antagonistic activity of *Bacillus* KF2 was most effective when the supernatant was obtained from 4 and 6-hour of cultures. The percentage decrease in the growth rate index was 80.9 and 80.7, respectively.

The results obtained for the other supernatants were also high and their impact on the growth of *Alternaria* sp. fluctuated in the 77-79 percent range. The lowest impact on the growth rate index had 24-hour bacterial culture. The value of the growth rate index amounted 0.98 and was almost 5-fold lower compared with the control test (Fig. 1).

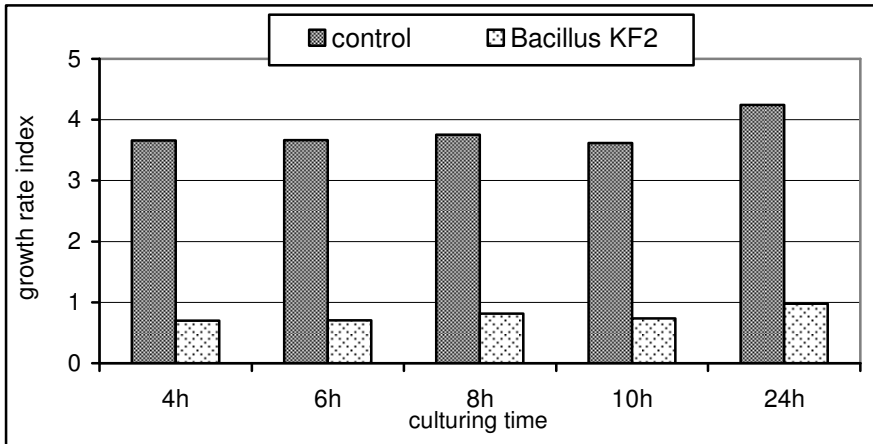


Fig. 1. Antifungal activity of *Bacillus* KF2 on Czapek medium against *Alternaria* sp.

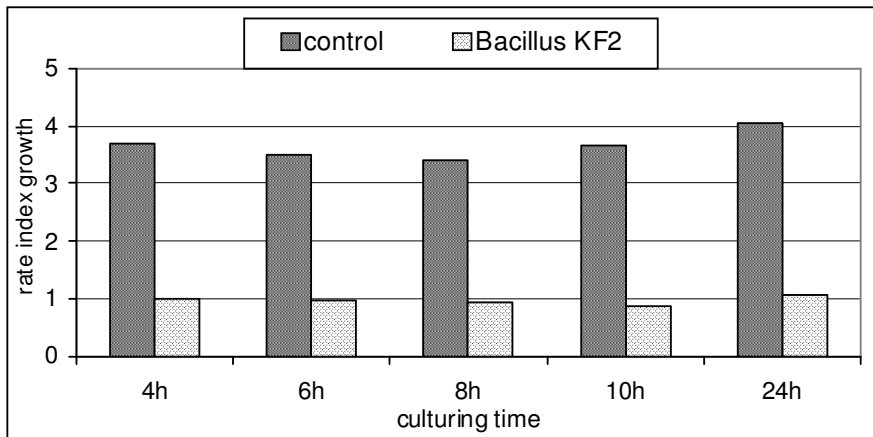


Fig. 2. Antifungal activity of *Bacillus* KF2 on PDA medium against *Alternaria* sp.

This strain proved to be equally efficient on the PDA medium as showed on Czapek medium limiting the linear growth of *Alternaria* sp. in the range from 72 to 76% when compared with the control. The most effective was the 10-hour culture whereas at least effective 8 hour culture of the *Bacillus* KF2 strain, where the index growth rate amounted 0.87 and 0.92, respectively (Fig. 2). On both media, the difference in the inhibitory action

of metabolites between the most and least acting culture of *Bacillus* KF2 amounted to slightly over 3.0%.

In presented study, fungistatic activity was also found for *Bacillus* BK2 against *Alternaria* sp. However, its effectiveness was significantly lower than that observed for strain *Bacillus* KF2. On PDA medium has been observed that with the raising the length of culturing time increased gradually fungistatic activity of this strain.

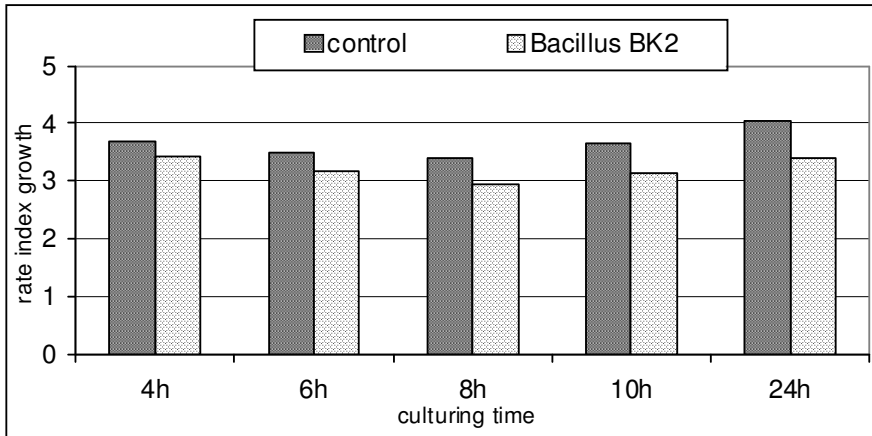


Fig. 3. Antifungal activity of *Bacillus* BK2 on PDA medium against *Alternaria* sp.

The decrease in the growth rate index was very low and amounted from 7.8 to 16.3% for the supernatants obtained from 4 and 6-hour culture, respectively. The linear growth of the mycelium was inhibited most actively when the medium was supplemented with 24-hour supernatant of *Bacillus* BK2 and amounted 3.40 compared with the control - 4.06 (Fig. 3).

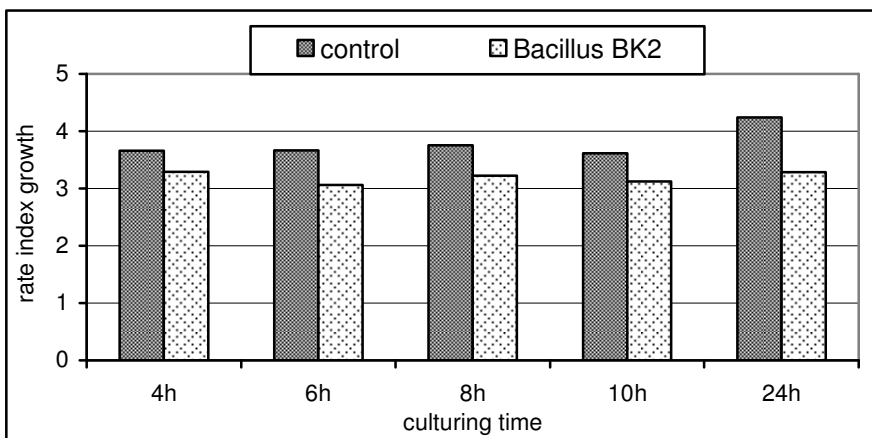


Fig. 4. Antifungal activity of *Bacillus* BK2 on Czapek medium against *Alternaria* sp.

Slightly higher activity of this strain was recorded on the Czapek-Dox medium. As the PDA medium, the growth rate inhibition of *Alternaria* sp. was the weakest when the supernatant of 4-hour culture of this strain was applied and amounted only 10%. However, 2-fold higher level of reduction in the growth rate was found for the supernatant obtained from the oldest namely 24 hour culture. The decrease of growth rate index achieved the value of 22.5% (Fig. 4).

On both media, the difference in the inhibitory action of metabolites between the youngest and oldest culture of *Bacillus* BK2 was 2-fold and amounted to about 10%. Although the growth of *Alternaria* sp. on the Czapek medium, both in the control and the test, was higher than on PDA medium, then the differences in the activity of these two strains were comparable.

### Conclusion

1. The inhibitory properties of *Bacillus* sp. strains were depended on the kind of the ones: the age of the bacterial culture and/or strains of *Bacillus*, the composition of the medium, duration of the experiment and the susceptibility of the fungus.
2. Supernatants obtained from the culture of both strains showed fungistatic activity against *Alternaria* sp., although the *Bacillus* KF2 showed a stronger effect than *Bacillus* BK2.
3. The growth of *Alternaria* sp. was strongly inhibited (from 72 to 81%) by *Bacillus* KF2 on both media, however by *Bacillus* BK2 to a lesser extent (from 7 to 22%).
4. The experimental results established the fungistatic activity of the tested *Bacillus* strains and indicate the possibility of using theirs, especially *Bacillus* KF2, as antifungal agents against *Alternaria* sp.

### References

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## AKTYWNOŚĆ PRZECIWGRZYBOWA BAKTERII Z RODZAJU *Bacillus* WOBEC *Alternaria* sp.

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**Abstrakt:** Celem podjętych badań była ocena aktywności fungistatycznej supernatantów otrzymanych z 4-, 6-, 8-, 10- i 24-godzinnych hodowli *Bacillus* KF2 i *Bacillus* BK2 wobec *Alternaria* sp. Na podstawie indeksu tempa wzrostu określono aktywność fungistatyczną szczepów *Bacillus* sp. na podłożach PDA i Czapka. Wyniki badań

wskazują, że tempo wzrostu *Alternaria* sp. na tych podłożach było 4-krotnie oraz 5-krotnie wolniejsze po zastosowaniu supernatantów uzyskanych z hodowli *Bacillus* KF2 niż w próbce kontrolnej. Maksymalną aktywność tego szczepu zanotowano po zastosowaniu 10-godzinnej i 4-godzinnej hodowli odpowiednio na podłożach PDA i Czapka. Natomiast aktywność przeciwgrzybowa szczepu *Bacillus* BK2 była znacznie mniejsza niż *Bacillus* KF2. Wraz ze wzrostem wieku hodowli *Bacillus* BK2 do 24 h obserwowano wzrost hamowania tempa wzrostu na obu podłożach (od 16 do 22%). Supernatanty otrzymane z hodowli obu szczepów wykazały działanie fungistatyczne wobec *Alternaria* sp., przy czym silniejszy wpływ wykazał *Bacillus* KF2, redukując tempo wzrostu o ok. 70-80% w zależności od wieku hodowli. Uzyskane wyniki badań potwierdzają aktywność fungistatyczną szczepów *Bacillus* sp. i wskazują na możliwość wykorzystania ich, szczególnie szczepu *Bacillus* KF2, jako środków przeciwgrzybiczych wobec *Alternaria* sp.

**Słowa kluczowe:** *Bacillus* sp., *Alternaria* sp., aktywność fungistatyczna, indeks tempa wzrostu