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# ANTIFUNGAL ACTIVITY OF *Bacillus* spp. AGAINST *Fusarium* spp.

## AKTYWNOŚĆ PRZECIWGRZYBOWA BAKTERII Z RODZAJU *Bacillus* spp. WOBEC *Fusarium* spp.

**Abstract:** The aim of the study was to assess the fungistatic activity of supernatants obtained from 4, 6, 8, 10 and 24-hour culture of *Bacillus* KF2 and *Bacillus* BK2 against *Fusarium* spp. The antagonistic activity was evaluated on the basis the rate index of fungal growth on Czapek-Dox and PDA media. The rate index of *Fusarium* spp. growth on PDA medium was 5-fold and 3.5-fold slower than in the control, after application of supernatants obtained from the 24-hour culture (respectively for *Bacillus* KF2 and *Bacillus* BK2). Similarly, a high antifungal activity of the tested strains was observed on Czapek-Dox medium. The growth of *Fusarium* spp. was 6-fold and 3.5-fold slower after application of supernatants obtained from 6-hour culture, respectively, for *Bacillus* KF2 and *Bacillus* BK2. Supernatants obtained from the culture of both strains showed fungistatic activity against *Fusarium* spp., although the *Bacillus* KF2 strain showed a stronger impact than *Bacillus* BK2 strain. The inhibitory properties of *Bacillus* species was depended on the age of the bacterial culture and/or strains of *Bacillus*, and the composition of the medium. The experimental results exhibit the fungistatic activity of *Bacillus* strains and indicate the possibility of use their as antifungal agents against *Fusarium* spp.

**Keywords:** *Bacillus* spp., antifungal activity, *Fusarium* spp.

*Bacillus* spp. and relatives or their metabolites present an ecofriendly alternative to use of synthetic chemicals for plant growth enhancement in many different applications. Extensive research has demonstrated that these microorganisms could have an important role in agriculture and horticulture in improving crop productivity [1-3]. Direct mechanisms of these bacteria include the provision of bioavailable phosphorus for plant, nitrogen fixation, production of siderophores, production of phytohormones like cytokinins, auxins and gibberellins. Indirect mechanisms used by *Bacillus* spp. include reduction of iron available to phytopathogens bacteria, synthesis of fungal cell wall-lysing enzymes (cellulase, chitinase, β-1,3 glucanase), antibiotic production (phospholipides, lipoprotein) and protection against pathogenic fungi. The major antibiotics that play a vital role in the suppression of plant pathogens by *Bacillus* spp. (eg *B. cereus*, *B. subtilis*, *Paenibacillus polymyxa*, *B. circulans*, *B. coagulans*) are grouped into non-volatile (eg lipopeptides: iturins, fengycins, surfactins) and volatile antibiotics (eg hydrogen cyanide) [2, 4-8]. These metabolites have antibacterial and antifungal activity against phytopathogenic microorganisms (eg *Rhizoctonia* spp., *Pythium* spp., *Aspergillus* spp., *Botritis cinerea*, *Sclerotina sclerotinum*) [1, 9-11]. Antibiotics production is strongly conditioned by factors such as the strain microorganism, chemical composition of the medium and the incubation conditions (incubation time, agitation, pH and temperature). The main purpose of the study was to assess the antifungal activity of the *Bacillus* strains KF2 and BK2 on *Fusarium* spp.

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## 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 filtrate of these strains to inhibit of hyphal growth of *Fusarium* spp. The *Bacillus* strains were inoculated into a flask containing the nutrient broth and incubated at 30°C for various times to give 4, 6, 8, 10 and 24-hour culture (working culture). The *Fusarium* spp. strain was cultivated on Czapek-Dox medium at 25°C for 5 days. Each experiment was run in triplicate. 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 $cm^3$ (mL) 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* (*Ifg*) using the formula [12]:

$$Ifg = \frac{A}{D} + \frac{b_1}{d_1} + ... + \frac{b_x}{d_x}$$

where $A$ is the mean from colony measurement, $D$ is the experiment duration (days), $b_1...b_x$ is the increase a colony diameter from lasted measurement, $d_1...d_x$ is the number days from lasted measurement.

## Results and discussion

The antifungal activity of *Bacillus* strains KF2 and BK2 grown on 2 different media was evaluated towards tested *Fusarium* spp. as the rate index of fungal growth. The antifungal activity of these strains was depended on the strains of *Bacillus* spp. and also age of the culture applied and the growth media used. This might be due to the secretion of metabolites produced by these strains, diffused and dissolved into the culture media. Figure 1 shows the results obtained on the Czapek-Dox medium.

In this experiment 4, 6, 8, 10 and 24-hour culture of the *Bacillus* strains were used as an inhibition factor. It was observed that the rate index of *Fusarium* spp. growth was the slowest after application of the supernatants obtained from 6-hour culture compared with the control, around 72÷82%, for *Bacillus* BK2 and *Bacillus* KF2 respectively. Whereas the 24-hour culture of both these strains showed a very little inhibitory properties, although in case of *Bacillus* KF2 it was a quite high and amounted to 73%, but in case of *Bacillus* BK2 amounted only 9%.

The high antifungal activity the tested strains was also observed on PDA medium (Fig. 2).

It is appear that inhibitory activities of *Bacillus* KF2 was higher than *Bacillus* BK2. The growth of *Fusarium* spp. was strongly inhibited by both these strains, when were applied as 24-hour culture and amounted to 70÷78%, for *Bacillus* strains BK2 and KF2 respectively. However the *Bacillus* BK2 showed very little inhibitory properties as a 6-hour culture and even small stimulatory properties in case of 4-hour culture. *Fusarium*

spp. was also the less sensitive to metabolites produced by 4-hour culture of *Bacillus* KF2, and the suppress of its growth was still a quite big (about 59%) compared with the control.

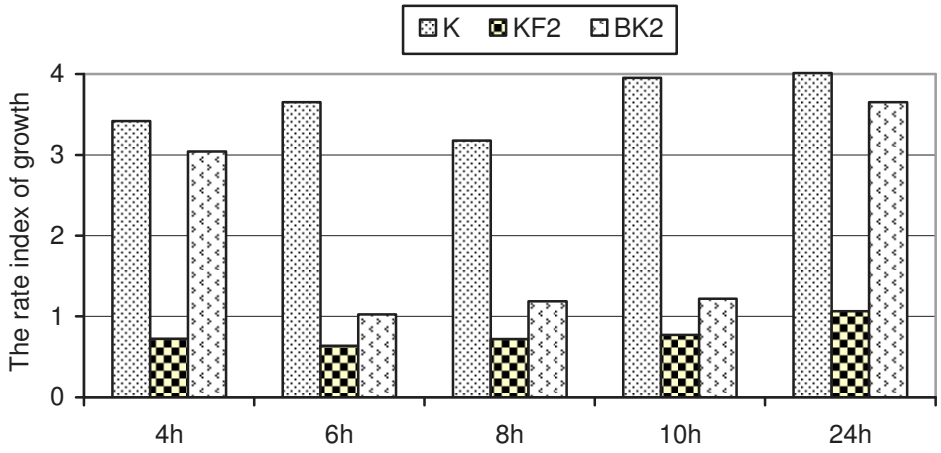


Fig. 1. Antifungal activity of *Bacillus* KF2 and *Bacillus* BK2 on Czapek-Dox medium against *Fusarium* spp.

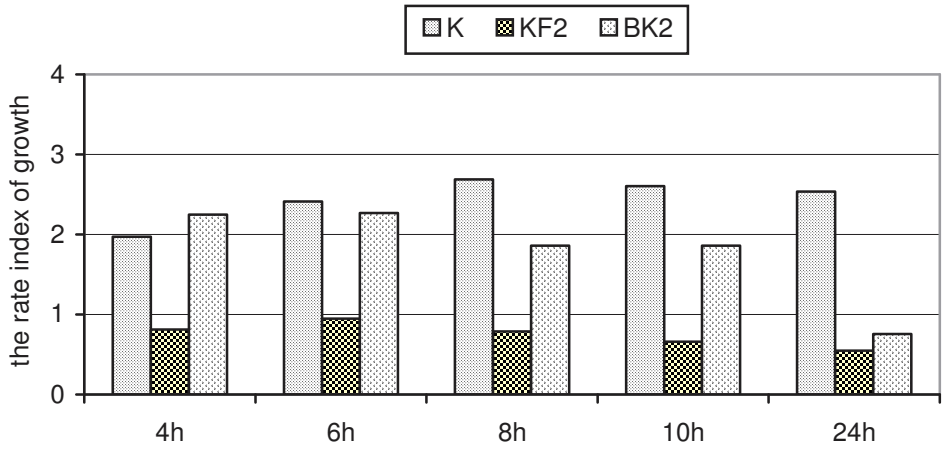


Fig. 2. Antifungal activity of *Bacillus* KF2 and *Bacillus* BK2 on PDA medium against *Fusarium* spp.

With these observations agree some authors, who evidenced that the different sensitivities of the fungi to the various *Bacillus* genus might be due to the secretion of secondary metabolites at different concentrations and dependent on the fungi membrane composition. Besides, the appearance of secondary metabolites in bacterial cultures is often confined to a certain growth phase. Production of most antibiotics begins by the end of the log phase and occurs throughout the stationary phase [11, 13].

Therefore, an interest of these microorganisms is increasing and possibility theirs application as biological control agent are investigated extensively and conducted in many countries around the world [1, 3, 4].

## Conclusions

1. The inhibitory properties of *Bacillus* species was depended on the kind of the ones: the age of the bacterial culture and/or strains of *Bacillus*, and the composition of the medium.
2. Supernatants obtained from the culture of both strains showed fungistatic activity against *Fusarium* spp, although the *Bacillus* KF2 strain showed a stronger effect than *Bacillus* BK2 strain.
3. The growth of *Fusarium* spp. was strongly inhibited on PDA medium by 4-hour culture of both *Bacillus* strains, while on Czapek-Dox medium by 6-hour culture.
4. The experimental results demonstrated the fungistatic activity of the tested *Bacillus* strains and indicate the possibility of using theirs as antifungal agents against *Fusarium* spp.

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# AKTYWNOŚĆ PRZECIWGRZYBOWA BAKTERII Z RODZAJU *Bacillus* spp. WOBEC *Fusarium* spp.

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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 *Fusarium* spp. Ocenę właściwości antagonistycznych metabolitów bakteryjnych przeprowadzono metodą hodowlano-płytkową z zastosowaniem podłoży Czapka i PDA. Tempo wzrostu *Fusarium* spp. na podłożu PDA było 5-krotnie oraz 3,5-krotnie wolniejsze niż w próbce kontrolnej, po zastosowaniu supernatantów uzyskanych z 24-godzinnej hodowli, odpowiednio dla *Bacillus* KF2 i *Bacillus* BK2. Podobnie dużą aktywność przeciwgrzybową badanych szczepów stwierdzono na podłożu Czapka. Indeks tempa wzrostu *Fusarium* spp. był 6-krotnie oraz 3,5-krotnie wolniejszy po zastosowaniu supernatantów z 6-godzinnej hodowli, odpowiednio dla *Bacillus* KF2 i *Bacillus* BK2. Supernatanty otrzymane z hodowli obu szczepów wykazały działanie fungistatyczne wobec *Fusarium* spp. przy czym silniejszy wpływ wykazał *Bacillus* KF2. Stopień zahamowania wzrostu *Fusarium* spp. zależał od gatunku bakterii, wieku jej hodowli, rodzaju podłoża i czasu trwania hodowli. Uzyskane wyniki badań wykazały aktywność fungistatyczną szczepów *Bacillus* spp. i wskazują na możliwość wykorzystania ich jako środków przeciwgrzybiczych wobec *Fusarium* spp.

**Słowa kluczowe:** *Bacillus* spp., aktywność przeciwgrzybowa, *Fusarium* spp.