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EFFECT OF *Bacillus subtilis* EXOMETABOLITES ON THE GROWTH RATE OF *Rhizoctonia solani*

WPŁYW METABOLITÓW *Bacillus subtilis* NA TEMPO WZROSTU *Rhizoctonia solani*

Abstract: The aim of the research was to assess a potential biological activity of cell-free supernatants (CFS) obtained from 4, 6, 8, 10 and 24-hour culture of *Bacillus subtilis* against 4 pathogenic strains of *Rhizoctonia solani* marked as R1, R2, R3 and R4. The antagonistic properties of metabolites were assayed by the dual-culture technique on PDA medium. Fungistatic activity of *B. subtilis* was determined on the rate of mycelia growth inhibition. On the basis of obtained results, it has been proved that fungistatic activity of *B. subtilis* is varied and depends on the age of the bacterial culture and susceptibility of the fungus. Taking into consideration all the analyzed parameters, *R. solani* R1 was the most sensitive but R3 lest sensitive to the metabolites produced by *B. subtilis.* The highest inhibition of the growth rate was obtained for *R. solani* R1 and the decrease of index amounted between 72-87%, whereas a lower inhibition was noted for strain R3 (44-78%), depending on the age of the bacterial cultures. *B. subtilis* may find a wide range of application in the process of plant protection against diseases caused by *R. solani.*

Keywords: *Bacillus subtilis*, *Rhizoctonia solani*, antifungal activity

Plant diseases caused by fungi as *Rhizoctonia solani* constitute the most numerous and the significant group of diseases taking into account an economic aspects. *Rhizoctonia solani* and other pathogenic *Rhizoctonia* species cause pre- and post-emergence damping-off of beet sugar, which can reduce seedling stands and yield. They can survive over winter and between sugar beet crops as sclerotia and melanized mycelium. Under conditions of high temperature and high soil moisture can cause seedling blight and subsequently brown root rot [1, 2]. 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. In recent years, there has been a growing interest in potential use as plant protection non-pathogenic microorganisms, such as bacteria of *Bacillus* kind, as promising alternatives to synthetic chemicals. They are an eco friendly and generally safe microorganisms that are ubiquitous in nature [3-5]. One of the most promising means to achieve this is the use new tools based on biological agents (BCAs) for disease control. Biological control agents interact with phytopathogens directly or indirectly to reduce the population of pathogens or reduction in the ability of the pathogens to cause disease. *Bacillus* spp. strains developed different mechanisms which limit pathogenic activity of fungi and show antifungal activity with varying degrees of antagonism. Several studies have found that some strains of the genus *Bacillus* eg. *B. megaterium*, *B. pumilus,*

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B. cereus. B. subtilis have a broad spectrum of activities and were reported to suppress the growth of pathogens such as: *Pythium* spp., *Fusarium* spp., *Aspergillus* spp. *Botritis cinerea* [4, 6-10]. Antagonistic properties of the *Bacillus* spp. is strongly conditioned by factors such as the strain of the bacteria, the chemical composition of the medium and the incubation conditions (the incubation time, temperature, aeration) as well as susceptibility of fungi. Therefore, the possibility of controlling soil-borne pathogens by introducing specific antagonistic bacteria to infested soil has been extensively investigated during the past decades [4, 6, 7, 11-14].

Therefore the aim of conducted research was to determine how metabolites produced by strain *Bacillus subtilis* affect the growth *Rhizoctonia* strains.

Materials and methods

The involvement of antifungal activity compounds produced by *Bacillus subtilis* strain in the inhibition of fungal growth was confirmed by the ability of cell-free culture filtrate of these strain to inhibit of hyphal growth of *Rhizoctonia solani* strains marked as R1, R2, R3 and R4.

Fungistatic activity of *B. subtilis* was determined with the culture-plate method on PDA medium containing glucose as carbon source. Fungal mycelial-discs (diameter of 10 mm) obtained from growing cultures of tested fungal isolates were placed in the centre of PDA medium containing 0,5 ml working cultures obtained from 4, 6, 8, 10 and 24-hour culture of *Bacillus subtilis*. The control plate contained *R. solani* cultures and aseptic broth medium in place of the supernatant. All plates were incubated at 26 ±2°C for 4-6 days and the diameters of fungal growth was measured every days. The experiment was performed in triplicate, where one repeat was represented by a one plate containing the growth medium with one mycelia disc. The influence of metabolites produced by *B. subtilis* on the growth of *R. solani* strains was determined as the growth rate index as described previously [15]. The inhibition of fungal growth was evaluated as the percentage reduction of the growth rate index in the treated plate versus the growth rate index in the control plate.

Results and discussion

Bacillus spp. can occur in the plant rhizosphere soil and exercise an antagonistic and competitive effect on the fungal communities. They have ability to produce biologically active compounds or plant growth regulators, which seem to play a major role in biological control of plant pathogens [3]. The mechanisms of antagonism of the biocontrol strains against the pathogens included competition for nutrients or space, production several different non-volatile and volatile of antimicrobial compounds, mycolytic enzymes, induction of defence responses in plant [3, 7, 11]. In this study the antifungal activity of *B. subtilis* was evaluated against 4 pathogenic strains of *Rhizoctonia solani* as the growth rate index (Figs. 1 and 2). The *Rhizoctonia* spp. strains showed different sensitivities and responses to the metabolites produced by *Bacillus subtilis* depending on the age of the culture. In this experiment 4, 6, 8, 10 and 24-hour culture of *B. subtilis* were used as an inhibitory factor. It was observed that the linear growth of the mycelium of *R. solani* R1 and R2 on PDA medium was inhibited most efficient by adding supernatants from 8-10 hours bacterial cultures to the growth medium compared to the control. The value of

the growth rate index of strains R1 and R2 amounted 1.19 and 2.09, respectively (Fig. 1). The highest reduction of the growth rate index was noted for *R. solani* marked as R1 and obtained reduction amounted 87%. Whereas the highest degree of inhibition the strain R2 was 11% lower than the strain R1 and achieve the value about 76% (Fig. 3).

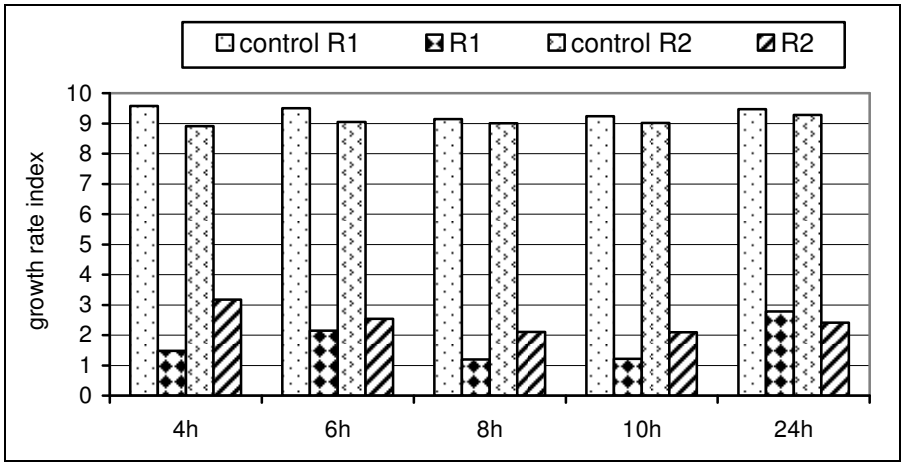

Fig. 1. The influence of *Bacillus subtilis* on mycelial growth of *Rhizoctonia solani* strains R1 and R2 on PDA medium

Significantly smaller effect of the tested bacteria on the growth of fungi was found in the case other strains of *R. solani* (Fig. 2).

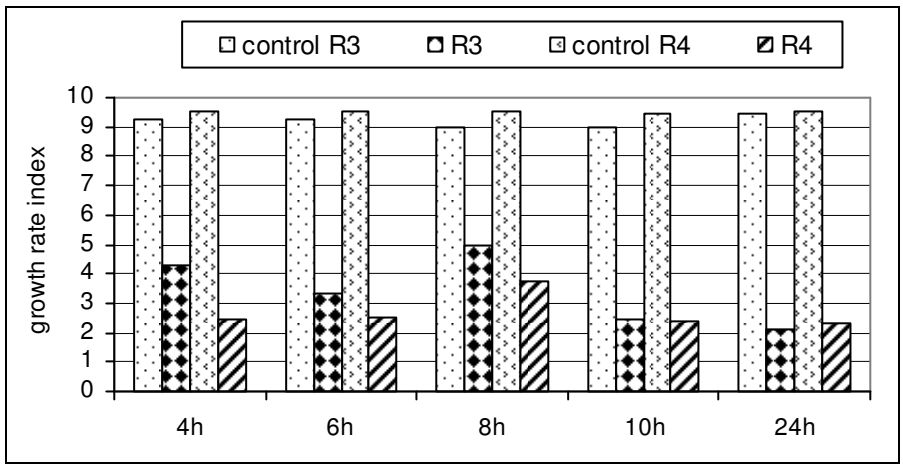

Fig. 2. The influence of *Bacillus subtilis* on mycelial growth of *Rhizoctonia solani* strains R3 and R4 on PDA medium

The growth of strains marked as R3 and R4 was strongly inhibited, when *B. subtilis* was applied as 24-hour supernatants, where the index growth rate amounted to 2.08-3.30, respectively. The range of percentage inhibition varied from 76-78 for this strains. The lowest reduction in the growth rate index was obtained in case of *R. solani* marked as R3 and achieved the value between 44-78% compared with the control (Fig. 3).

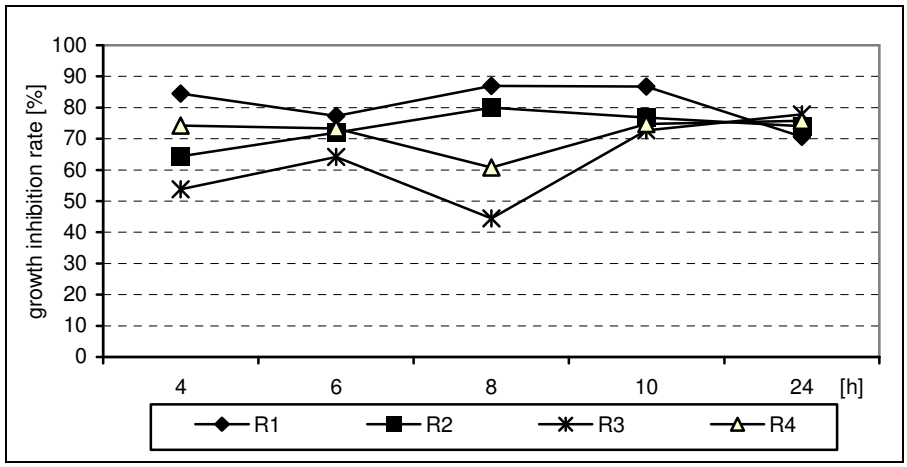

Fig. 3. Inhibition effect of *B. subtilis* on mycelial growth of *R. solani* strains

The largest differences in the effects of metabolites of *B. subtilis* on growth of *R. solani* was observed after application of 8-hour culture. The percentage inhibition of the growth rate index varied from 44 to 87. Whereas, the most similar inhibitory effects against all the tested strains *Rhizoctonia* spp. was observed after application suppernatants of 24-hours of culture *B. subtilis.* The inhibition of the growth of these fungi ranged from 71 to 78 percent (Fig. 3).

These inhibitive activities of *B. subtilis* against the hyphal growth of *R. solani* may be due to the production of hydrolytic enzymes, that can degrade structure of cell wall viz. β-1,3 glucanase, chitinase, protease and/or secretion of several cyclic lipopeptides, diffused and dissolved into the culture media. Some of this bioactive substances can cause induce hyphal deformation, enlargement of cytoplasmic vacuoles and cytoplasmic leakage, inhibit sclerotial germination of *R. solani.* Moreover most of these compounds are produced at the late growth stage or at logarithmic growth phase then they lost its antibiotic efficiency in stationary phase [3, 4, 7, 11].

Conclusions

Conducted research confirmed fungistatic properties of *Bacillus subtilis* against *Rhizoctonia solani* strains and prove that growth inhibition of the fungi depends not only on the biological properties and age of the bacterial culture and also susceptibility of the fungus to bacterial metabolites. Taking into consideration all the analyzed parameters, *R. solani* R1 was the most sensitive but R3 lest sensitive to the metabolites produced by

B. subtilis. The highest decrease of index was obtained for *R. solani* R1 and amounted between 72-87%, whereas a lower decrease of index was noted for strain R3 (44-78%). These studies confirm the sensitivity the different strains of *R. solani* on the metabolites secreted by *B. subtilis*. Therefore, this bacterium may found application as an antifungal agent, to protect of plants against diseases caused by *Rhizoctonia solani.*

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WPŁYW METABOLITÓW *Bacillus subtilis* NA TEMPO WZROSTU *Rhizoctonia solani*

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Abstrakt: Celem badań była ocena biologicznej aktywności supernatantów otrzymanych z 4-, 6-, 8-, 10- i 24-godzinnej hodowli *Bacillus subtilis* wobec 4 szczepów *Rhizoctonia solani* oznaczonych jako R1, R2, R3 i R4. Antagonistyczne właściwości metabolitów *B. subtilis* oznaczono metodą hodowlano-płytkową na podłożu PDA i oceniono na podstawie indeksu tempa wzrostu. Z uzyskanych wyników badań wykazano, że fungistatyczna aktywność *B. subtilis* jest zróżnicowana i zależy od wieku hodowli bakteryjnej oraz wrażliwości grzybów. Biorąc pod uwagę wszystkie analizowane parametry, szczep *R. solani* R1 był najbardziej wrażliwy, natomiast R3 najmniej na metabolity produkowane przez *B. subtilis*. Największe zahamowanie indeksu tempa wzrostu, w wysokości 72-87%, stwierdzono w przypadku *R. solani* R1, natomiast najmniejsze w przypadku szczepu R3 (44-78%) w zależności od wieku hodowli bakterii. *B. subtilis* może znaleźć szerokie zastosowanie w ochronie roślin przed chorobami wywołanymi przez *R. solani*.

Słowa kluczowe: *Bacillus subtilis*, *Rhizoctonia solani,* aktywność przeciwgrzybowa