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# THE APPLICATION OF *BACILLUS GLOBISPORUS* STRAIN ISOLATED FROM COMPOSTS OF FAT WASTES IN PLANT PROTECTION*

**Summary.** The study investigated the effect of a *Bacillus globisporus* strain and produced supernatants on growth of selected plant pathogens, i.e. *Alternaria alternata*, *Botrytis cinerea*, *Cladosporium* spp., *Trichothecium roseum*, *Fusarium oxysporum*, *F. solani*, *Trichoderma viride*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Verticillium dahliae.* The highest fungistatic activity of bacterial cultures was found against *R. solani*, *S. sclerotiorum*, *B. cinerea* and *T. roseum*, whereas in case of the supernatant, it was found against *R. solani* and *S. sclerotiorum*. At identical concentrations, the fungistatic activity of *B. globisporus* culture appeared stronger than that of the supernatant. No significant effect of bacterial culture concentration in the medium was observed. Growth of *Cladosporium* spp., *V. dahliae*, *T. viride*, and *A. alternata* was found both after seven and 10 days of the experiment. In case of the supernatant, this situation was observed only for *F. oxysporum*, after seven days.

**Key words:** *Bacillus globisporus*, plant protection, biological activity, plant pathogens

## Introduction

In the investigations on the applications of biological methods in plant protection, an increasing number of studies have recently been conducted on fungistatic properties of

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composts produced from sewage sludge and different plant materials. These days, a new field emerges in biotechnology. This field concerns plant protection and consists in the controlled inoculation of composts with beneficial microorganisms capable of inhibiting the growth of many phytopathogens. It is believed that this technology needs to be developed on a commercial scale, since composts constitute for an ideal source of nutrients for mass-scale production of active microflora in biological protection (PHAE et AL. 1990, HOITINK et AL. 1991, GREBUS et AL. 1993, HOITINK et AL. 1997).

Bacteria from the genus *Bacillus* have been applied with increasing frequency in plant protection against both pests and plant diseases (JAYARAJ et AL. 2005, SZCZECH and SHODA 2006, BACON and HINTON 2007). Literature data indicate that numerous strains of *B. subtilis* are capable of synthesizing different biologically active compounds, i.e. lytic enzymes of antibiotic proteins, including those of a volatile character (PHAE et AL. 1990, PODILE and PRAKASH 1996, FOLKMAN et AL. 2003).

These investigations were conducted on a strain of *B. globisporus*, isolated from composts from fat wastes (Piotrowska-Cyplik 2009, unpublished data). This study investigated the effect of *B. globisporus* strain culture and culture liquid on growth of selected plant pathogens.

## Material and methods

### Marker strains

Fungistatic action of the strain of *B. globisporus* was tested against ten plant pathogens: *Alternaria alternata*, *Botrytis cinerea*, *Cladosporium* spp., *Trichothecium roseum*, *Fusarium oxysporum*, *F. solani*, *Trichoderma viride*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Verticillium dahliae*. Marker fungi were cultured on Petri dishes on solid PDA medium (Merck) until abundant mycelium growth was obtained (approximately 10 days). Thereafter, the corks with mycelium were cut with the use of a cork borer for the purpose of determinations.

### Test strains

The experiment was conducted on a strain of *Bacillus globisporus*. Growth medium for test strains: $K_2HPO_4$ – 3 g/l, $KH_2PO_4$ – 3 g/l, $(NH_4)_2SO_4$ – 2 g/l, $MgSO_4 \times 7H_2O$ – 0.5 g/l, $CuSO_4 \times 5H_2O$ – 0.2 g/l, $FeSO_4 \times 7H_2O$ – 0.035 g/l, $H_3BO_3$ – 0.3 g/l, $MnSO_4 \times 5H_2O$ – 0.2 g/l, $CoSO_4 \times 7H_2O$ – 0.025 g/l, $ZnCl_2$ – 0.105 g/l, fat substance – rapeseed oil 20 g/l, distilled water 1 $dm^3$.

### Method of determination of fungistatic activity of *Bacillus globisporus* strains

Fungistatic activity of the tested strain was determined by the plate method as described by BORECKI (1984). In test cultures, a 48-h *B. globisporus* culture was introduced to PDA medium at 1%, 3%, and 10% (v/v), as well as culture liquid – the supernatant, introduced at 3%, 5% and 10% (v/v). Culture liquid was obtained after the isolation of bacterial biomass (centrifugation at 10 000 rpm) and sterilization through microbiological filters not binding the proteins (Millipore 0.22 μm).

The control comprised cultures of marker fungi on media, which contained distilled water instead of cell biomass culture or the supernatant. All plate cultures were run at a temperature of 37°C for 14 days. After seven and 10 days, the percentage of growth inhibition of marker fungi was determined according to the formula: $(K_0 - F/K_0)100$, where: $K_0$ – diameter of culture in the control combination, $F$ – diameter of culture in the test combination.

Statistical analysis of the experiments was conducted by employing Duncan's test at a significance level of 0.05 and the Bliss transformation separately for the culture and the supernatant at individual dates.

## Results and discussion

Results of the experiment presented in Tables 1 and 2 indicate that all the tested pathogens were susceptible to the action of the *B. globisporus* strain culture and the produced supernatant as well. The growth of pathogens in the presence of the culture and the supernatant added to the medium at maximum concentrations was inhibited within the range from 49 to 89%. The highest fungistatic activity of the bacterial culture was found against *R. solani*, *B. cinerea*, *S. sclerotiorum* and *T. roseum*; while that of the supernatant, towards *R. solani* and *S. sclerotiorum*.

Table 1. The influence of *Bacillus globisporus* culture on the growth of indicator fungi (% of growth inhibition)
Tabela 1. Wpływ hodowli *Bacillus globisporus* na wzrost grzybów wskaźnikowych (% zahamowania wzrostu)

| Indicator fungi | After 7 days | | | After 10 days | | |
|---|---|---|---|---|---|---|
| | concentration of culture in medium | | | | | |
| | 1% | 3% | 5% | 1% | 3% | 5% |
| *Alternaria alternata* | 71.2 c | 72.7 cd | 71.2 c | 78.4 hi | 78.5 hi | 78.5 hi |
| *Botrytis cinerea* | 83.1 e | 85.4 fg | 88.2 fg | 71.2* cd | 82.5* fgh | 83.6* j |
| *Cladosporium* spp. | 51.2 a | 51.3 a | 51.2 a | 68.4 bc | 71.6 cd | 71.6 cd |
| *Trichothecium roseum* | 82.3 e | 86.4 f | 83.2 e | 72.2* de | 79.0* i | 76.0* ghi |
| *Fusarium oxysporum* | 59.2 b | 59.4 b | 66.1 c | 57.5 b | 61.6 cd | 71.4 fg |
| *Fusarium solani* | 78.7 d | 79.2 e | 81.4 e | 62.3* b | 73.1* efg | 76.1* fgh |
| *Trichoderma viride* | 78.6 e | 78.8 e | 79.1 e | 82.3 i | 82.4 i | 73.6 fgh |
| *Rhizoctonia solani* | 85.0 fg | 86.3 g | 86.5 g | 84.2* j | 88.6* j | 89.0* j |
| *Sclerotinia sclerotiorum* | 49.0 a | 69.2 g | 67.2 fg | 50.2* a | 85.8* j | 86.2* j |
| *Verticillium dahliae* | 61.5 b | 61.5 b | 61.6 b | 75.5 efg | 73.2 ef | 74.4 efg |

*The lower inhibition of pathogen growth after 10 than 7 days arose due to limitation of fungi growth by dishwall in culture control.

Mean values marked with the same letter do not differ significantly at $\alpha = 0.05$.

Table 2. The influence of supernatant from *Bacillus globisporus* culture on the growth of indicator fungi (% of growth inhibition)
Tabela 2. Wpływ supernatantu z hodowli *Bacillus globisporus* na wzrost grzybów wskaźnikowych (% zahamowania wzrostu)

| Indicator fungi | After 7 days | | | After 10 days | | |
|---|---|---|---|---|---|---|
| | concentration of supernatant in medium | | | | | |
| | 3% | 5% | 10% | 3% | 5% | 10% |
| *Alternaria alternata* | 56.1 ef | 69.8 hi | 71.8 ij | 70.2 fgh | 78.6 kl | 81.3 kl |
| *Botrytis cinerea* | 63.6 fgh | 79.2 lmn | 81.0 no | 64.1 cde | 77.4* jk | 83.5* l |
| *Cladosporium* spp. | 38.1 a | 42.3 ab | 53.2 bc | 59.2 abc | 63.6 cd | 65.8 def |
| *Trichothecium roseum* | 67.3 fgh | 69.8 klmn | 68.4 jklmn | 54.2* a | 72.6* fghi | 68.8* efg |
| *Fusarium oxysporum* | 54.57 cd | 55.31 fg | 54.9 gh | 58.6 ab | 72.5 fg | 75.4 ghij |
| *Fusarium solani* | 73.8 jkl | 78.4 jklm | 80.0 lmn | 64.2* cde | 73.2* fghi | 74.3* ghij |
| *Trichoderma viride* | 65.6 fg | 73.7 ijk | 76.7 jkl | 61.6 bc | 77.2 ijk | 77.3 ijk |
| *Rhizoctonia solani* | 75.7 jklmn | 86.4 p | 87.2 p | 74.4* hij | 85.4* m | 85.5* m |
| *Sclerotinia sclerotiorum* | 81.5 mn | 84.5 op | 87.3 p | 80.2 kl | 85.2 m | 87.1* m |
| *Verticillium dahliae* | 45.8 b | 57.2 de | 61.5 efg | 57.8 ab | 66.5 efg | 74.3 ghij |

*The lower inhibition of pathogen growth after 10 than 7 days arose due to limitation of fungi growth by dishwall in culture control.

Mean values marked with the same letter do not differ significantly at $\alpha = 0.05$.

Growth inhibition amounting to 80% or more for the lowest concentration of the culture in the medium, i.e. 1% (v/v), was recorded for *R. solani*, *B. cinerea* and *T. roseum*. In case of the lowest concentration of the supernatant in the medium, i.e. 3% (v/v), such a strong action was observed only in relation to *S. sclerotiorum*. At the same concentration – 3% (v/v), the fungistatic activity of the *B. globisporus* culture turned out to be stronger than that of the supernatant. A higher fungistatic potential of vegetative forms of cells in the tested strain indicates a complex mechanism of their action, connected most probably with antibiosis (FOLKMAN et AL. 2003), and also with the phenomenon of competition. As a result of the competition, most typically for nutrients, plants are colonized, i.e. infested by the conquering microorganism. It is believed that the capacity for effective colonization of leaf surfaces, roots, etc., is the basis for effective biocontrol (MARI et AL. 1996). Bacteria from genus *Bacillus* are applied against plant pathogens mainly in form of spores; however, biological results of such an application may be weaker when the vegetative forms are used (COLLINS and JACOBSEN 2003).

In the experiment, no significant effect was found for the concentration of the bacterial culture applied in the substrate on the growth of the following pathogens: *Cladosporium* spp., *V. dahliae*, *T. viride* and *A. alternata*, when evaluated both after seven and 10 days. In case of the supernatant, such a situation was observed only for *F. oxysporum* when evaluated after seven days.

The high fungistatic activity of the *B. globisporus* strain against major plant pathogens as well as the natural occurrence of these bacteria in compost and soil might sug-

gest their application in plant protection. This protection concerns not only soil-borne diseases but also diseases connected with underground parts and seeds of plants, caused by *R. solani*, *B. cinerea*, *Fusarium* spp., *S. sclerotiorum* and *T. roseum*.

The strong action of the investigated strain in relation to *B. cinerea* and the spore-formulating capacity of tested bacteria make them potentially suitable for foliar applications. *Bacillus subtilis* turned out to be effective in the limitation of the occurrence of *Cercospora beticola* on sugar and red beets (COLLINS and JACOBSEN 2003), whereas *B. subtilis* strain QST 713 may be applied in the control of several diseases of aboveground parts of plants (THE MANUAL... 2004). The potential site of *B. globisporus* application may also be the stored plant organs attacked by fungi from genera *Rhizoctonia*, *Botrytis* and *Trichothecium*.

The potential applications of *B. globisporus*, presented above, are limited to fungal and fungi-like pathogens. Literature data also indicate that bacteria from genus *Bacillus* are active against bacterial pathogens, e.g. *Xanthomonas campestris* pv. *campestris* (WULFF et AL. 2002) and *Ervinia amylovora* (THE MANUAL... 2004). In case of the tested strain, this problem will be investigated in course of further investigations.

## Concluding remarks

*Bacillus globisporus* could be widely applied in plant protection against fungal and fungi-like diseases connected with the substrate and underground parts of plants, as well as seeds, and against diseases of the aboveground parts of plants and those attacking plant organs stored in warehouses.

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## ZASTOSOWANIE SZCZEPU *BACILLUS GLOBISPORUS* WYIZOLOWANEGO Z KOMPOSTÓW Z ODPADÓW TŁUSZCZOWYCH W OCHRONIE ROŚLIN

**Streszczenie.** Badania dotyczyły wpływu hodowli szczepów *Bacillus globisporus*, jak również otrzymanego supernatantu, na wzrost wybranych patogenów roślinnych: *Alternaria alternata*, *Botrytis cinerea*, *Cladosporium* spp., *Trichothecium roseum*, *Fusarium oxysporum*, *F. solani*, *Trichoderma viride*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Verticillium dahliae.* Największą aktywność fungistatyczną hodowli bakteryjnej stwierdzono wobec *R. solani*, *S. sclerotiorum*, *B. cinerea* i *T. roseum*, a supernatantu – wobec *R. solani* i *S. sclerotiorum*. Na poziomie tego samego stężenia aktywność fungistatyczna hodowli *B. globisporus* okazała się silniejsza niż supernatantu. W doświadczeniu stwierdzono brak istotnego wpływu stosowanego stężenia hodowli bakteryjnej w podłożu na wzrost następujących patogenów: *Cladosporium* spp., *V. dahliae*, *Trichoderma viride* i *A. alternata* zarówno po siedmiu, jak i 10 dniach hodowli. W przypadku supernatantu taką sytuację obserwowano jedynie u *F. oxysporum* po siedmiu dniach hodowli.

**Słowa kluczowe:** *Bacillus globisporus*, ochrona roślin, aktywność biologiczna, patogeny roślinne

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