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BIOCONTROL ACTIVITIES OF *Pseudomonas fluorescens* AGAINST ASPARAGUS PATHOGEN

AKTYWNOŚĆ BIOLOGICZNA *Pseudomonas fluorescens* WOBEC PATOGENA PORAŻAJĄCEGO SZPARAGI

Abstract: *Pseudomonas* spp. and their metabolites present environmentally friendly alternative to chemical products to improve plant growth in many different applications. Extensive studies have shown that these microorganisms and their metabolites could have an important role in agriculture and horticulture in improving crop productivity. The aim of the research was to assess a potential biological activity of *Pseudomonas fluorescens* against *Fusarium oxysporum* isolated from the spears of asparagus. The antagonistic properties of metabolites were assayed with a dual culture plate method on PDA and Czapek media for supernatants obtained from 6, 12, 24 and 48-hour culture of *P. fluorescens.* The culturing process was conducted at 26°C for 8 days and the fungal linear growth was measured every 1-2 days and compared to control. The fungistatic activity of *P. fluorescens* was estimate on the basis the growth rate index. The highest inhibition of the linear growth of mycelium, reaching 61%, has been found for 48-hour supernatants at OD = 2.0 and lowest for 12-hour supernatants on Czapek medium compared with the control trial. Significantly weaker linear growth of mycelium within the range of 4.0-33.0% was observed on PDA medium with a maximum inhibition for 48-hour supernatants at OD = 2.0 (33.0%). Promising method to asparagus protection against *Fusarium* sp. may be the use of *P. fluorescens* as the biocontrol agents.

Keywords: asparagus, *Fusarium oxysporum*, antifungal activity, *Pseudomonas fluorescens*

Introduction

Asparagus *(Asparagus officinalis* L.) is a vegetable (spears) grown worldwide, with well documented knowledge on its nutritional value. They are known as a source of vitamins, microelements, protein. It is essential for consumers to have asparagus spears free of pathogenic fungi and toxins produced by them. Fungi of *Fusarium* genus are one of the most significant pathogens of asparagus [1-3]. Some of the pathogens are able to form mycotoxins - secondary metabolites (*eg* fumonisin, moniliformin, trichothecenes) with possible health hazards and significant influence on food safety [1, 4, 5].

Fusarium oxysporum, *F. proliferatum* occurring and occasionally occured *F. culmorum and F. solani* in asparagus are known as the most important fungal pathogens worldwide causing crown and root rot of asparagus [1, 3, 6]. Asparagus plant protection is particularly difficult. Currently there are no safe chemicals for protection of asparagus against agrophages. Promising method to control of *Fusarium* spp. may be the biological resources of the group of bacterial antagonists. Therefore, *Pseudomonas* spp. and their metabolites may be an ecofriendly alternative to chemicals for plant growth enhancement. Many research has demonstrated that these microorganisms and their metabolites could have an important role in agriculture and horticulture in enhanced crop productivity. They may be useful as potential antagonist towards phytopathogenic fungi *eg*

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Rhizoctonia solani, *Verticillium* spp., *Aspergillus niger*, *Fusarium* spp. [7-12]. *Pseudomonas* species have been demonstrated to show antifungal activity with varying degrees of antagonism. The production of lytic enzymes (chitinase, glucanase, pectinase), salicylic acid, iron (Fe)-chelating siderophores, indole-3-acetic acid (IAA), HCN and secondary metabolites including antibiotics is probably one of the most important mechanisms of *Pseudomonas* antifungal properties. Many effective antibiotics synthesized by *Psudomonas* spp. has been detect, such as pyoluteorin (Plt), pyrrolnitrin (Prn) and phenazine-1-carboxylic acid (PCA) [11, 13-16]. In many instances the production of these compounds has been directly correlated with biocontrol activity [11]. Therefore, it is presently believed that *Pseudomonas* can play a significant role in biological control.

The purpose of this study was to evaluate the antifungal of *Pseudomonas fluorescens* against *Fusarium oxysporum* isolated from the asparagus.

Materials and methods

Due to the fact that many of the factors determine the activity of the microorganisms in the carried out tests taken into consideration three: the bacterial cell density, the composition of the medium and the cell-free supernatant. In this study the fungistatic properties of the *P. fluorescens* supernatants were determined against the growth rate index and the rate of mycelial growth inhibition of *Fusarium oxysporum.* The strain of tested fungus was isolated from the white spears of asparagus.

Mycelium growth. Fungistatic activity of *P. fluorescens* was determined with the culture-plate method on PDA and Czapek media. Fungal mycelial-discs (diameter of 7.0 mm) obtained from growing cultures of tested fungal isolates were placed in the centre of this media containing 0.5 cm^3 supernatants (inoculum) obtained from 6, 12, 24 and 48-hour culture of *P. fluorescens* at different optical density (OD = 1.0 and OD = 2.0). The control plate contained only *F. oxysporum* cultures and aseptic broth medium in place of the supernatant. All plates were incubated at 25 ±2°C for 8 days and the fungal linear growth was measured every 1-2 days until the mycelium of *F. oxysporum* in the control plate, reached the edge of the plate and compared to the control. The experiment was conducted in four replicates, where one repeat was represented by a one plate containing the growth medium with one mycelia disc. The influence of metabolites secreted by *P. fluorescens* on the growth of *F. oxysporum* was estimated as the growth rate index [17]. The inhibition of fungal growth was described 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

The involvement of antifungal activity compounds produced by *Pseudomonas fluorescens* in the inhibition of fungal growth was confirmed by the ability of cell-free culture filtrate of this strain to inhibit the hyphal growth of *F. oxysporum.* Conducted tests revealed the direct influence of *P. fluorescens* on the growth rate of the tested fungus under study. Studies shows that prolonging the bacterial culturing time and at the same time increasing the amount of secondary metabolites affect the inhibition the growth of the fungus.

On the PDA medium the reduction of the growth rate index increases as the culturing time of bacteria is longer. The measured values of the indexes for the control ranged between 56.6-59.9. However, in the trials tested the value of the growth rate index for the bacterial inoculum at initial optical density equal 1.0 amounted 38.4-57.5 and 38.4-46.9 (for OD equal 2.0) (Fig. 1).

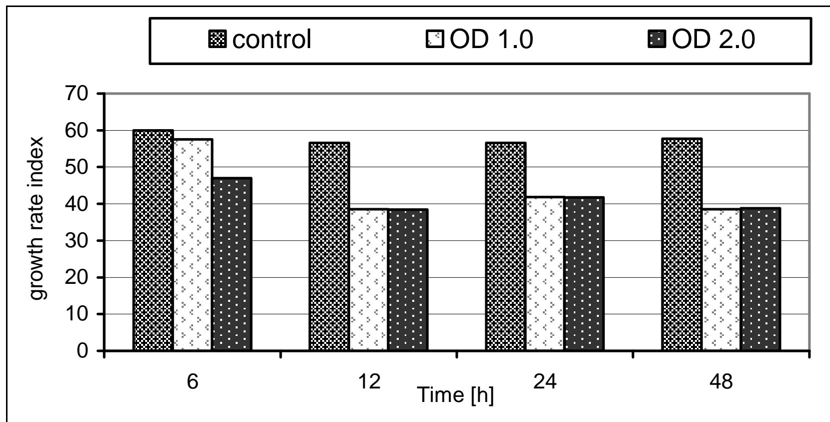

Fig. 1. Influence of *P. fluorescens* on the growth rate index of *F. oxysporum* on PDA medium

The highest inhibition of the growth rate index of *F. oxysporum* was noted in case of 48-hour culture of *P. fluorescens* at OD equal 2.0 (33.2%), while the lowest in case of metabolites obtained after 6-hours culture of this strain at OD equal 1.0 (3.98%) (Fig. 2).

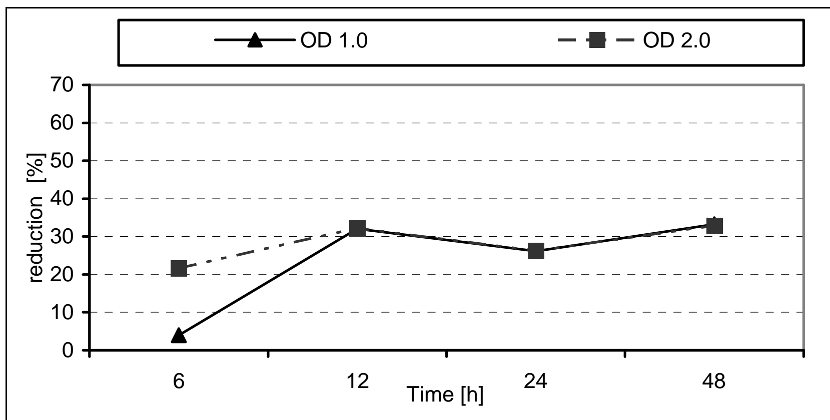

Fig. 2. Reduction of the growth rate index of *F. oxysporum* on PDA medium

The highest measured value of the growth inhibition indexes were noted on the medium with sucrose (Czapek medium). The value of the growth rate index amounted

21.8-27.6 (for OD equal 1.0) and 20.5-26.9 (for OD equal 2.0) compared to the control (54.8-63.2) (Fig. 3).

The most effective was the 48-hour culture of *P. fluorescens* at OD equal 2.0, while the lowest in case of 12-hour culture at OD equal 1.0. The decrease in the growth rate index amounted 61.4 and 51.4%, respectively. Despite everything they were the best results the influence of *P. fluorescens* on the *F. oxysporum* obtained during these tests (Fig. 4).

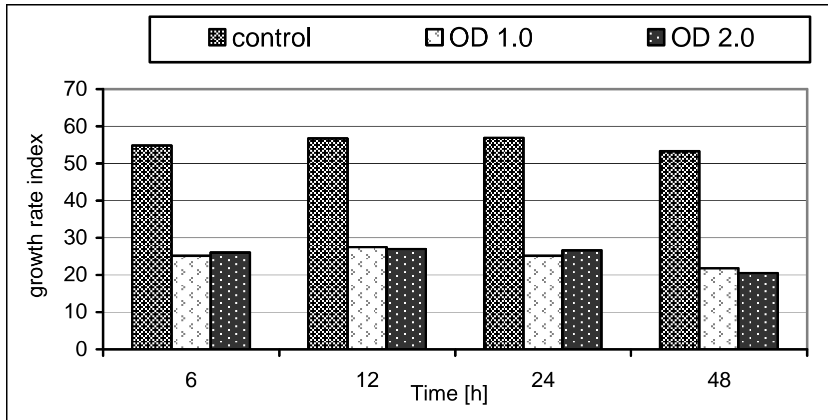

Fig. 3. Influence of *P. fluorescens* on the growth rate index of *F. oxysporum* on Czapek medium

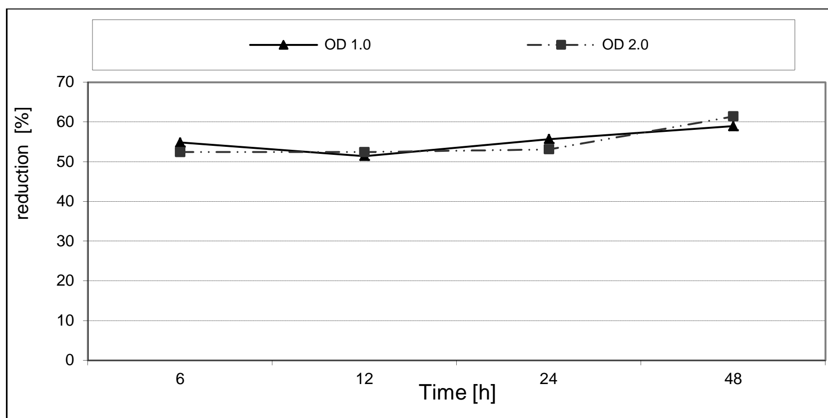

Fig. 4. Reduction of the growth rate index of *F. oxysporum* on Czapek medium

Selective activity of *P. fluorescens* against of phytopathogens has been described in many research papers. The researchers report that the antifungal properties of Pseudomonas largely depend on the capability of secretion of secondary metabolites, notably lytic enzymes and antibiotics [15, 18]. Koche et al [7] found that *P. fluorescens* isolate Pf_{20} was most efficient in inhibiting the mycelial growth up to 38.88%. Whereas, research by Toua

et al [8] demonstrated restricting the growth of the two formae speciales of *F.oxysporum* from 8.33 to 49.33% by five strains of *P. fluorescens.* Moreover conidial germinartion and germ tube elongation were inhibited and reduced. The results of the experiments by Jankiewicz et al [15] indicate the importance synthesized by *P. fluorescens* of salicylic acid in reducing of fungal phytopathogens. The least sensitive to salicylic acid proved to *F. graminearum*, and growth inhibition was found for *F. culmorum*. The inhibition of mycelial growth amounted to successively from 50% for *F. greaminarum*, next between 80-90% for *F. avenaceum* and *F. solani* and in the range 80-100%, depending on the tested *Pseudomonas* strain [15]. The inhibitory properties of *P. fluorescens* obtained in our research could be probably due to the production by own strain of secondary metabolites and/or lytic enzymes that can degrade cell wall. Although Jankiewicz [19] reported that fungal lysis need not necessarily be caused by lytic enzymes capable of decomposition of glycosidic bonds - chitinase and β 1,3 glucanase but also by other substances which are manufactured by bacteria from the *Pseudomonas* genus which include intensively secreted siderophores, hydrogen cyanide as well as exogenous proteases.

Conclusions

Conducted research confirmed fungistatic properties of *P. fluorescens* against *F. oxysporum* 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. Based on the data obtained in the these studies it can be concluded that the highest inhibition of the linear growth of mycelium, reaching 61%, has been observed for 48-hour supernatants on Czapek medium. A slightly weaker linear growth of mycelium have been observed on PDA medium with a maximal inhibition of 48-hour supernatants (33.0%). Therefore, promising method to asparagus protection against *Fusarium* sp. may be the application of *P. fluorescens* as the biocontrol agents. However, antagonist Pseudomonas with the antagonistic activity in vitro may not act in vivo due to environmental conditions and competition with other microorganisms. Therefore, it is important that biocontrol potential under field conditions should be further evaluated.

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AKTYWNOŚĆ BIOLOGICZNA *Pseudomonas fluorescens* WOBEC PATOGENA PORAŻAJĄCEGO SZPARAGI

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Abstrakt: *Pseudomonas* spp. oraz ich metabolity mogą stanowić alternatywę dla związków chemicznych w celu poprawy wzrostu roślin. Mogą one odegrać istotną rolę w rolnictwie i ogrodnictwie w poprawie wydajności upraw. Celem przeprowadzonych badań była ocena właściwości przeciwgrzybowych *Pseudomonas fluorescens* wobec *F. oxysporum*, wyizolowanego z podstawy pędów szparaga. Ocenę właściwości antagonistycznych metabolitów bakteryjnych (6-, 12-, 24- i 48-godzinnych) przeprowadzono metodą hodowlano-płytkową z zastosowaniem podłoży Czapka i PDA. Hodowlę prowadzono w temp. 26ºC przez 8 dni, dokonując pomiarów co 1-2 dni i porównywano w stosunku do kontroli. Na podstawie indeksu tempa wzrostu określono aktywność fungistatyczną *P. fluorescens*. Największą inhibicję wzrostu liniowego grzybni wynoszącą 61% obserwowano na podłożu Czapka po zastosowaniu supernatantów z 48-godzinnej hodowli bakterii o gęstości 2.0, a najmniejszą dla 12-godzinnej hodowli. Znacznie słabszą inhibicję wzrostu grzybni, w zakresie od 3,98 do 33,2%, obserwowano na podłożu PDA. Maksimum aktywności (33,0%) stwierdzono dla supernatantów pozyskanych z 48-godzinnej hodowli bakterii o gęstości 2.0. Efektywną metodą zmniejszenia porażenia szparagów przez *Fusarium* sp. może być zastosowanie bakterii *P. fluorescens* jako czynnika biologicznej ochrony.

Słowa kluczowe: szparagi, *Fusarium oxysporum*, aktywność przeciwgrzybowa, *Pseudomonas fluorescens*