DOI: 10.2429/proc.2014.8(1)008 2014;8(1)

Małgorzata NABRDALIK[1] and Katarzyna GRATA[1]

ANTIFUNGAL ACTIVITY OF *Pseudomonas fluorescens* AGAINST PHYTOPATHOGENIC STRAINS OF *Rhizoctonia solani*

AKTYWNOŚĆ PRZECIWGRZYBOWA *Pseudomonas fluorescens* WOBEC FITOPATOGENNYCH SZCZEPÓW *Rhizoctonia solani*

Abstract: The aim of conducted research was to determine the influence of metabolites of *Pseudomonas fluorescens* on the growth of 4 pathogenic strains of *Rhizoctonia solani* marked as R1, R2, R3 and R4 infesting sugar beet. The antagonistic properties of metabolites were assessed after 4, 6, 8, 10 and 24 hours of culturing of *P. fluorescens* with a culture-plate method on Czapek medium. The bacterial strains were cultured at 25ºC for 4-7 days. Fungistatic activity of *P. fluorescens* was determined on the rate of mycelial growth inhibition and of the growth rate index. Obtained results have proved that the strains of *Rhizoctonia* spp. under study were sensitive to *P. fluorescens* metabolites. The highest inhibition of the linear growth of fungi was noted for *R. solani* R1 and R3. In all cases the highest inhibition of the growth rate was obtained after 4 and 6 hours of culturing and the lowest was noted after 10 or 24 hours of culturing. After supplementing the growth medium of *P. fluorescens* the drop of the growth rate index was noticeable and reduction amounted between 78 and 89%. Conducted research confirmed fungistatic properties of *P. fluorescens* strains against *R. solani* strains. The tests showed that growth inhibition of the mycelium depends not only on the type of metabolites produced by a specific bacterial strain but also on the length of culturing.

Keywords: fungistatic activity, growth rate index, *Pseudomonas fluorescens*, *Rhizoctonia solani*

Introduction

Diseases of cultivated plants caused by *Rhizoctonia solani* Kühn are controlled mainly by an application of chemical substances or agronomic techniques (proper crop rotation, avoiding excessively moist and clumped soil) [1]. However, the residues of chemical substances in plants and the environment and the risk of pathogens' growing resistance to the treatment bring about the need to search for new protection methods. The methods include among others biological measures. According to the Directive 2009/128/EC of the European Parliament and of the Council, sustainable biological methods, employing microorganisms residing in a rhizosphere which are able to degrade toxins produced by pathogens, should be preferred to chemical methods.

The biological protection of plants includes different types of amensalism, especially antybiosis as well as a competition between protective microorganisms and pathogens for nutrients, energy and habitat [2]. When biological methods are considered, special attention is paid to PGPR microorganisms (*plant growth promoting rhizobacteria*) which produce enzymes performing hydrolysis of the cell wall of pathogenic fungi resulting in its degradation and consequently termination of the pathogens. PGPR microorganisms include bacteria such as: *Pseudomonas, Bacillus, Paenibacillus, Brevibacillus, Agrobacterium, Burkholderia, Pantoea, Lysobacter* [2-5].

[1] Independent Chair of Biotechnology and Molecular Biology, Opole University, ul. kard. B. Kominka 6a, 45-032 Opole, Poland, phone +48 77 401 60 56, email: mnabrdalik@uni.opole.pl
[*] Contribution was presented during ECOpole'13 Conference, Jarnoltowek, 23-26.10.2013

The aim of conducted research was to assess the usefulness of *Pseudomonas fluorescens* strain against phytopathogenic strains of *R. solani.*

Materials and methods

In the experiment, a fungistatic activity of *P. fluorescens* against 4 strains of *R. solani* marked R1, R2, R3 and R4 has been assessed. The strain *P. fluorescens* was isolated from soil and identified with the use of ID32GN tests (Biomerieux). The strains of tested fungi were isolated from the infested bulbs of sugar beetroot and diagnosed on the basis of their macro- and microscopic features.

The bacteria were cultured in the broth medium for 48 hours at 30°C. Next, the broth was inoculated with the suspension of 10^6 cfu/ml density and incubated for the time period from 4 to 24 hours. After the incubation it was centrifuged at 10 000 rpm and obtained supernatant underwent further analysis.

Conducted tests employed a culture-plate method applied on Czapek growth medium consisting of [g/dm^3]: sucrose 30.0, $MgSO_4 \times 7H_2O$ 0.5, KH_2PO_4 1.0, KCl 0.5, $NaNO_3$ 3.0, $Fe_2(SO_4)_3 \times 7H_2O$ 0.01, agar 15.0. Tested growth media were inoculated with supernatants obtained after 4, 6, 8, 10 and 24 hours of culturing of *P. fluorescens* rods. Next, the media were inoculated with 10mm discs overgrown with 7-days old mycelium of tested *R. solani* strains. The control trials contained only tested *R. solani* strains with no addition of supernatant. All plates were incubated at 25ºC for 5 days. The diameters on the plates were measured every day until the mycelium of *R. solani,* in the control trial, reached the edge of the plate. The experiment was conducted in 6 trials, where one trial was represented by one culturing plate with the growth medium and the mycelial disc.

The influence of metabolites produced by *P. fluorescens* on the growth of *R. solani* strains was determined against the growth rate index, calculated according to the formula [6]:

$$T = \frac{A}{D} + \frac{b_1}{d_1} + \frac{b_2}{d_2} + \ldots + \frac{b_x}{d_x}$$

where: T - growth rate index, A - mean value of diameter measurements [mm], D - the length of the experiment (number of days), b_1, $b_2 \ldots b_x$ - increase in a diameter size since the last measurement, d_1, $d_2 \ldots d_x$ - number of days since the last measurement.

The fungistatic properties of the supernatant have been assessed on the basis of the linear growth inhibition of the fungus.

Results and discussion

In the conducted research, presented in this paper, strains *Pseudomonas fluorescens* were tested in terms of their applicability to control phytopathogenic strains of *R. solani*. Laboratory tests allowed to determine the direct influence of metabolites produced by *P. fluorescens* on the growth pace of tested fungi illustrated by calculated growth rated indexes and an inhibition of the mycelial growth (Figs. 1-3).

The analysis of the mycelial growth of *Rhizoctonia* spp. strains showed that in all control trials its pace was very fast in time (Fig. 1).

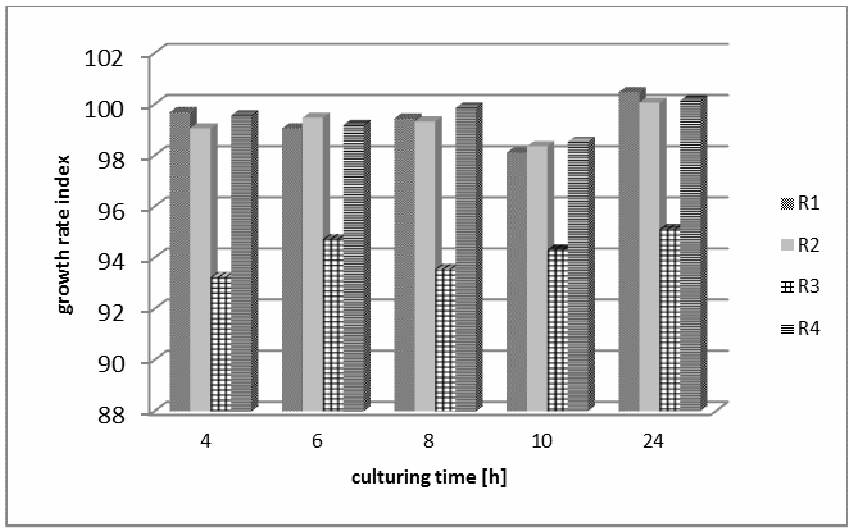

Fig. 1. The growth rate index of *R. solani* for the control trials

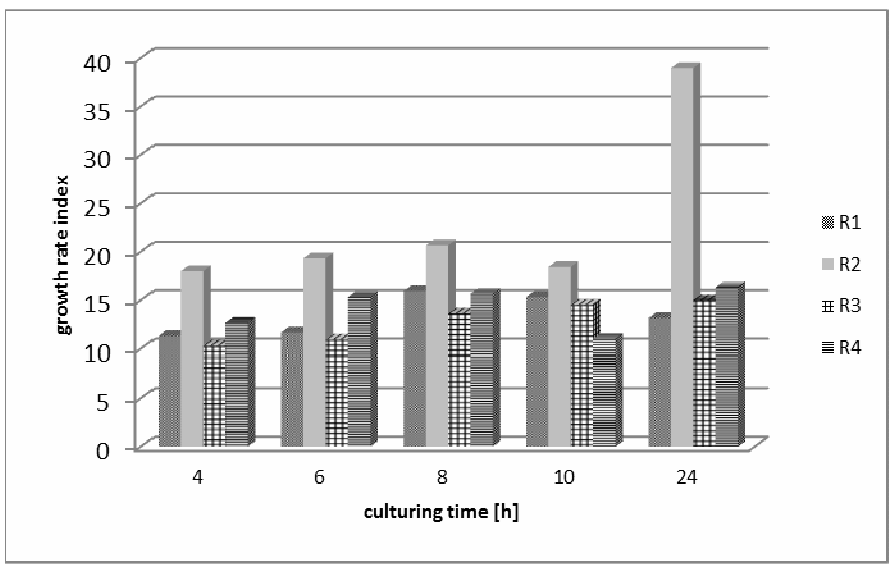

Fig. 2. The growth rate index of *R. solani* for the proper trials

The plate with growing medium was fully covered with the fungus after 3 days in case of the strains: R1, R2 and R4, for which the value of the growth rate index ranged between 98.11 and 100.47. The highest measured values were obtained after 24 hours of culturing and the lowest after 10 hours of culturing. The recorded values of the growth rate index were similar after 4, 6 and 8 hours of culturing and amounted around 99 units. The control strain, marked R3 covered the whole surface of the plate after 4 days of culturing.

Therefore, in this case the values of the growth rate index were lower and fluctuated between 93.24 and 95.11 after 4 hours and 24 hours of culturing respectively.

Metabolites produced by *P. fluorescens* were collected after 4, 6, 8, 10 and 24 hours of an incubation process and then added to the growing media which significantly affected an inhibition of the growth rate index of tested *R. solani* strains (Fig. 2). Regardless of the culturing time of *P. fluorescens* and the type of the fungi strains applied, obtained results of the growth rate index were lower in comparison with the results recorded for the control trials. The results presented in Figure 3, prove unquestionably that the highest inhibition of the growth rate index has been obtained after the application of metabolites obtained after 4 and 6 hours of bacteria culturing, except for the strain R2, for which the most effective were metabolites produced after 4 hours and 10 hours. The strain *P. fluorescens* applied in the research was the most active in case of the strains: R1 and R3 and the recorded value of the reduction of the growth rate index amounted over 88% after the application of the metabolites produced after 4 and 6 hours of the incubation. It seems that the same bacterial strain was least active in case of the strain R2, although the reduction of the growth rate index amounted between 79.19 and 83.18% (Fig. 3).

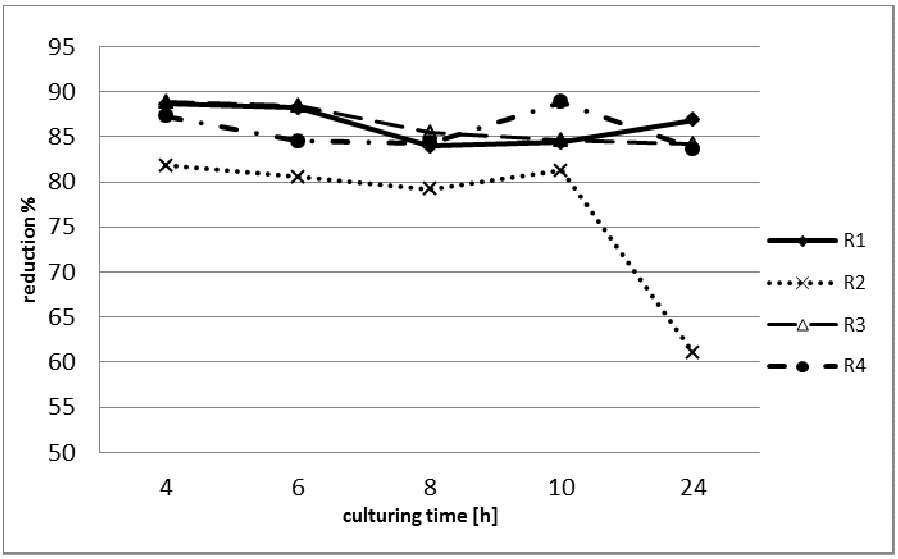

Fig. 3. Influence of *P. fluorescens* on the linear growth rate index of tested fungi *R. solani*

The analysis of obtained results prove that each tested strain of *R. solani* showed a different reaction towards applied metabolites of *P. fluorescens* obtained at its different growth phase. The bacterial strain under study did not inhibit the growth rate index of *R. solani* completely but only restricted it to the range of 78.24-88.95%. Therefore the question has arisen of whether obtained results allow to classify *P. fluorescens* as a useful strain in the process of biological protection against Rhizoctonia diseases? Although many authors [4, 7-10] confirm antagonistic activity of *P. fluorescens* strains towards *R. solani*, conducted tests should be treated as pilot research which requires further confirmation.

Conclusions

1. Obtained results show significant efficiency of *P. fluorescens* against phytopathogenic strains of *R. solani.*
2. The differences concerning the growth inhibition degree noted in the research for individual strains allow to state that the process depends on the strain itself and the length of culturing process.
3. The highest antifungal activity was noted after an application of the supernatants of *P. fluorescens* obtained after 4 and 6 hours of culturing, which corresponds with the early phase of the logarithmic growth.
4. Significant differences obtained during the tests and lack of complete growth inhibition of *R. solani* prove that further large-scale laboratory tests including different strains of *P. fluorescens* should be conducted prior to field tests.

References

[1] Moliszewska EB. Etiologia wybranych chorób korzeni buraka cukrowego. Studia i Monografie 412. Opole: Wyd Uniwersytetu Opolskiego; 2009.
[2] Pal KK, McSpadden Gardener B. The Plant Health Instructor. 2006. DOI: 10.1094/PHI-A-2006-1117-02.
[3] Nagarajkumar M, Jayaraj J, Muthukrishnan S, Bhaskaran R, Velazhahan R. Microbiol Res. 2005;160:291-298. DOI: 10.1016/j.micres.2005.02.002.
[4] Brewer MT, Larkin RP. Crop Protection. 2005;24:939-950. DOI: 10.1016/j.cropro.2005.01.012.
[5] Gwiazdowski R, Gwiazdowska D, Ostrowska A. Progress in Plant Protection. 2011;51:3:1261-1264.
[6] Burgieł Z. Acta Agrar et Silvestr Ser Agraria. 1984;23:187-199.
[7] Hammer PE, Hill DS, Lam ST, Van Pée KH, Ligon JM. Appl Environ Microbiol. 1997;63:2147–2154.
[8] Corbell N, Loper JE. J Bacteriol. 1995;177:6230-6236.
[9] Sumner DR, Lewis JA, Gitaitis RD. Crop Protection. 1992;11:121-126. DOI: 10.1016/0261-2194(92)90093-K.
[10] Nabrdalik M, Grata K. Proc ECOpole. 2012;6(2):541-545. DOI: 10.2429/proc.2012.6(2)073.

AKTYWNOŚĆ PRZECIWGRZYBOWA *Pseudomonas fluorescens* WOBEC FITOPATOGENNYCH SZCZEPÓW *Rhizoctonia solani*

Samodzielna Katedra Biotechnologii i Biologii Molekularnej, Uniwersytet Opolski

Abstrakt: Celem podjętych badań było określenie wpływu metabolitów *Pseudomonas fluorescens* na wzrost 4 fitopatogennych szczepów buraka cukrowego *Rhizoctonia solani* oznaczonych jako R1, R2, R3 oraz R4. Ocenę właściwości antagonistycznych metabolitów przeprowadzono metodą hodowlano-płytkową na podłożu Czapka dla 4-, 6-, 8-, 10- i 24-godzinnych hodowli *P. fluorescens*. Hodowle prowadzono w temperaturze 25ºC przez 4-7 dni. Na podstawie stopnia zahamowania wzrostu grzybni oraz indeksu tempa wzrostu określono aktywność fungistatyczną *P. fluorescens*. Wyniki doświadczenia wskazują, że wśród badanych szczepów *Rhizoctonia* spp. były szczepy wrażliwe na działanie metabolitów *P. fluorescens*. Największą inhibicję rozrostu liniowego grzybni zaobserwowano dla szczepów *R. solani* R1 oraz R3. W obu przypadkach najwyższe zahamowania wzrostu grzybni uzyskano dla 4- i 6-godzinnej hodowli, a najniższe dla hodowli 10- i 24-godzinnej. Po wprowadzeniu do podłoża hodowli *P. fluorescens* zaobserwowano spadek indeksu tempa wzrostu, uzyskując redukcję od około 78 do 89%. Przeprowadzone badania potwierdzają fungistatyczne działanie szczepów *P. fluorescens* wobec szczepów *R. solani*. Przeprowadzone analizy wykazują, że inhibicja wzrostu grzybni uzależniona jest nie tylko od rodzaju metabolitów wydzielanych przez dany szczep bakterii, ale również od wieku jej hodowli.

Słowa kluczowe: aktywność fungistatyczna, indeks tempa wzrostu, *Pseudomonas fluorescens*, *Rhizoctonia solani*