

## ANTAGONISM OF SPORULATING *BACILLUS* AND *CLOSTRIDIUM* BACTERIA AND FLUORESCENT *PSEUDOMONAS* BACTERIA ISOLATED FROM UNDER MAIZE WITH PATHOGENIC *FUSARIUM* SPECIES ON DIFFERENT CULTURING MEDIA

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**Abstract.** There are numerous reports regarding *in vitro* production by bacteria of antifungal and antibacterial metabolites which were found to act also *in vivo*. Substances exudated by microorganisms are characterised by the ability to exert an antagonistic influence on other microbes and to induce a systemic resistance in plants. The above-mentioned antagonism encompasses the following three kinds of interactions between organisms: amensalism, competition for food and place and parasitism. On the basis of the current state of knowledge as well as research results, the above-mentioned bacterial properties are increasingly frequently employed in biological plant protection. The objective of this research project was to isolate from the soil under maize cultivation bacteria which are mycolytic in relation to the following three species of fungi: *Fusarium culmorum*, *Fusarium oxysporum* and *Fusarium poae*. The strains were obtained from the Department of Phytopathology of Poznań University of Life Sciences. Bacteria of antifungal properties were isolated from the soil under maize cultivation onto media selective for *Pseudomonas*, *Bacillus* and *Clostridium* genera. Mycolytic activity of the isolated strains was tested on different substrates: potato dextrose agar (PDA) and peptone-agar. The obtained research results revealed that the mycolytic properties of the isolated strains depended on the type of the applied substrate. Sensitivity of fungi against the isolated bacterial genera was always recorded on the peptone-agar substrate in contrast to the PDA substrate on which no such correlation was observed.

**Key words:** mycolysis, *Fusarium*, *Pseudomonas*, *Bacillus*, *Clostridium*, PDA medium, peptone-agar medium

### INTRODUCTION

A growing interest has been observed in recent years in biological preparations based on non-pathogenic soil microorganisms. Biopreparations can provide an alternative to pesticides due to their high effectiveness [Jankiewicz i Kuzawińska 2009]. Chemical plant protection agents (fungicides, insecticides and pesticides) applied so far, apart from the elimination of crop plants pathogens, can also disturb biological balance in the environment or exert a negative influence on consumers' health [Zydlik 2008, Niewiadomska et al. 2012].

Evolving interests in environmental issues increase proportions of alternative control methods of plant pests in relation to those applied until recently. Novel protection proposals of cultivations offer utilisation of preparations containing in their composition live preparations (biological preparations) such as: fluorescent bacteria from the *Pseudomonas* genus, bacteria from the *Bacillus*

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and *Clostridium* genera as well as others. There are numerous reports claiming that biological preparations of *Pseudomonas fluorescens* can control effectively fungal diseases in orchard production, among others, the fungus that causes apple scab [Škvarčuk 2000] or development of fungal diseases of plums and sour cherries [Ševčuk et al. 2004]. On the other hand, bacteria from the *Bacillus* genus restrict the development of fungal apple root disease [Sharma 2002].

Looking for appropriate strains of antifungal bacteria, it is worth focusing attention on the bacteria from the *Pseudomonas* genus as well as sporulating bacteria from the *Bacillus* and *Clostridium* genera which occur abundantly and actively in the rhizosphere of crop plants. It is evident from the literature on the subject that strains of these bacteria developed various mechanisms constraining disease causing activities of fungal pathogens. These mechanisms include, among others: synthesis of lytic enzymes, antibiotics and, additionally, in the case of *Pseudomonas fluorescens*, also siderophores and hydrogen cyanide [Handelsman and Stabb 1996, Nagarajkumar et al. 2004].

The objective of this study was to isolate bacteria of antifungal properties from the soil from under maize cultivation and to investigate their antagonistic properties in relation to the following three species of fungi: *Fusarium cullmorum*, *Fusarium oxysporum* and *Fusarium poae* on two different culturing media: potato-dextrose agar (PDA) and peptone-agar medium.

## MATERIAL AND METHODS

Bacteria of antifungal properties were isolated in the years 2007–2008 from the soil under maize cultivation of the Experimental Station in Złotniki which belongs to Poznań University of Life Sciences. The collected soil samples were used to isolate bacterial strains using the method of surface inoculation according to Koch [Kunicki-Goldfinger 2001] after securing earlier soil dilutions of  $10^7$ . King B medium was used to culture the isolated antagonistic bacteria. Generic identification was performed on the basis of API tests, microscopic analyses additionally employing Gram staining and – in the case of fluorescence bacteria from the *Pseudomonas* genus – using a fluorescence lamp of 365 nm wave length.

Strains of fungal pathogens: *Fusarium cullmorum*, *Fusarium oxysporum* and *Fusarium poae* were obtained from the Department of Phytopathology of Poznań University of Life Sciences.

The antagonistic activities of three the isolated bacterial strains against fungi were investigated on two kinds of bacterial media. For this purpose, 4 ml of physiological salt were added aseptically to passaged *Fusarium* strains incubated on Burholder's medium and next, the obtained suspension in the amount of 1 ml was poured onto Petri dishes and flushed with an appropriate PDA or peptone agar medium. The mixture was thoroughly mixed and incubated in a thermostat at the temperature of 25°C for 24 h. Next, on the obtained "mist" of fungal culture growth, a 2-day old isolated strain of bacteria was applied onto the centre of the Petri dish and incubated at the temperature of 25°C. After 72-h culturing, macroscopic and then microscopic evaluation was performed. The assessment involved evaluation whether any antagonistic response of bacteria against the fungus in the form of fungal growth took place. After the macroscopic evaluation, impression preparations were made and, using a Nikon light microscope, a photographic documentation was prepared.

## RESULTS AND DISCUSSION

Strains of sporulating bacteria of *Bacillus* and *Clostridium* genera as well as fluorescent bacteria of *Pseudomonas* genus isolated from the rhizosphere zone in "in vitro" conditions were

characterised by strong antagonism in relation to cereal phytopathogens from the *Fusarium* genus only on peptone-agar medium (Tab. 1). In the case of the PDA medium, totally reverse results were obtained; bacteria from *Bacillus* and *Pseudomonas* genera exhibited positive taxis against fungal pathogens from the *Fusarium* genus, while bacteria from the *Clostridium* genus turned out to be neutral (Tab. 2).

Table 1. Antagonistic activity of isolated bacterial strains in relation to fungal phytopathogens

Fungus	Growth inhibition of the fungus on peptone-agar medium		
	<i>Pseudomonas</i> sp.	<i>Bacillus</i> sp.	<i>Clostridium</i> sp.
<i>Fusarium cullmorum</i>	+	+	+
<i>Fusarium oxysporum</i>	+	+	+
<i>Fusarium poae</i>	+	+	+

“+” – positive mycolytic effect

Table 2. Stimulating activity of isolated bacterial strains in relation to fungal phytopathogens

Fungus	Stimulation of fungal growth on PDA medium		
	<i>Pseudomonas</i> sp.	<i>Bacillus</i> sp.	<i>Clostridium</i> sp.
<i>Fusarium cullmorum</i>	+	+	-
<i>Fusarium oxysporum</i>	+	+	-
<i>Fusarium poae</i>	+	+	-

“+” – stimulation; “-” – absence of stimulation

Similar results were reported by Benizir et al. [1995] who demonstrated, among others, that the mycolytic activity of bacteria from the *Pseudomonas* genus depended on the kind of medium on which the antagonistic activity against the fungal pathogen was examined. In addition, they also found that in the case of the peptone-agar medium, the examined bacteria exhibited antifungal action as a result of manufacture of antibiotics, volatile compounds and siderophores and not as a result of production of lytic enzymes. However, they failed to record such changes on the PDA medium.

Also Jankiewicz [2010] reported that fungal lysis need not necessarily be caused by lytic enzymes capable of decomposition of glycosidic bonds – chitinase and  $\beta$  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.

In the performed investigations, the strongest antifungal activity was determined in the case of fluorescent bacteria from the *Pseudomonas* genus in relation to three species of pathogens from the *Fusarium* genus. Antagonistic activities of *Pseudomonas* bacteria against some species of microscopic fungi have already been described in the literature on the subject. At the present

time, investigations are focused on the recognition of interdependence mechanisms between antagonistic microorganisms and plant pathogens.

In the case of the mycolytic action of fluorescent bacteria from the *Pseudomonas* genus, colour changes in the medium into orange were observed (Fig. 1). This could have been caused by the secretion by this strain of pyoverdinin, which is a typical siderophore manufactured by fluores-

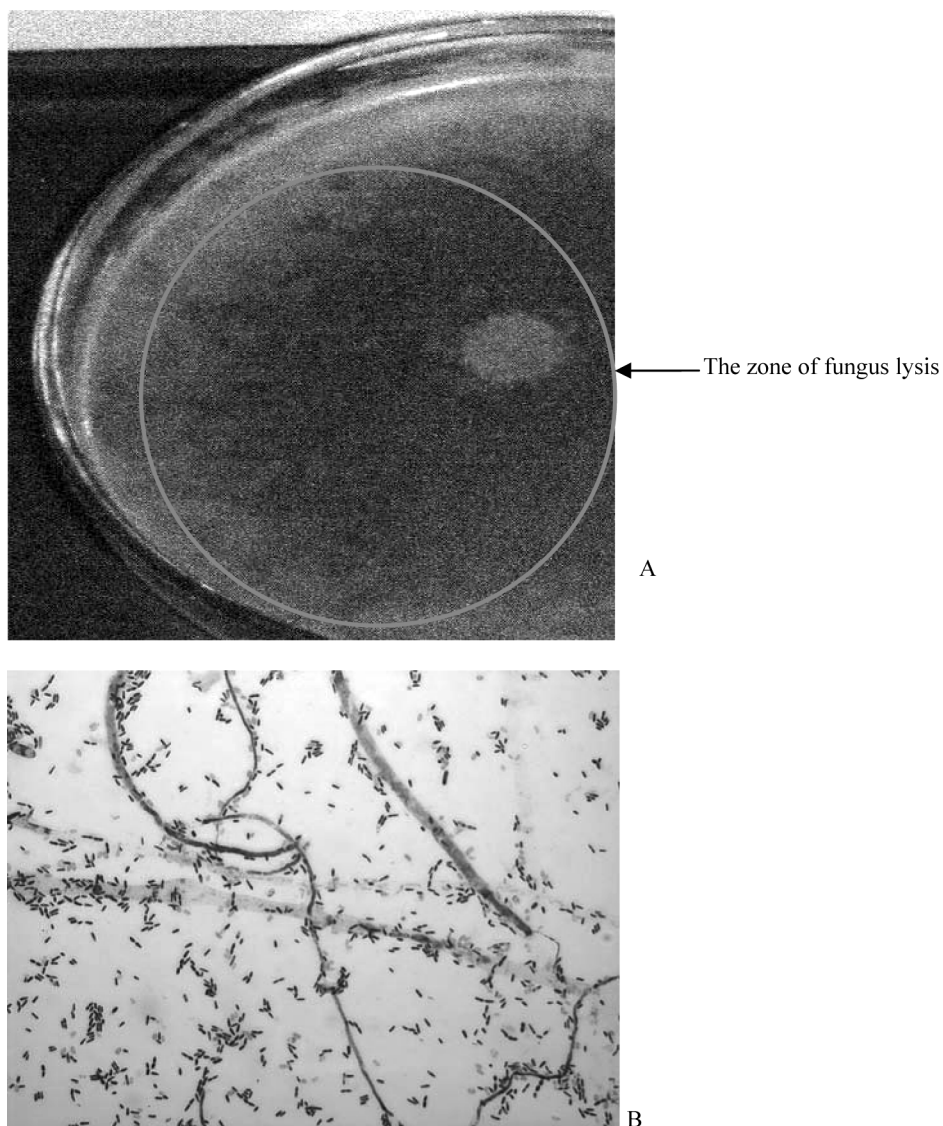


Fig. 1. Antagonistic properties of fluorescent *Pseudomonas* bacteria on peptone-agar medium against pathogenic fungi from *Fusarium* genus. A – macroscopic image; B – microscopic image

cent *Pseudomonas* bacteria. According to Fuchs et al. [2001] and Jankiewicz and Kuzawińska [2009], pyoverdinin is a very effective tool which inhibits growth of phytopathogens because it exhibits affinity of iron III ions. This property confines the access of the controlled microorganism to iron.

In the performed experiment, in the case of sporulating bacteria from the *Bacillus* genus and *Clostridium* (Fig. 2 and 3), mycolytic activities were also observed on peptone-agar medium with respect to the examined pathogens from the *Fusarium* genus but areas of growth inhibition were smaller.

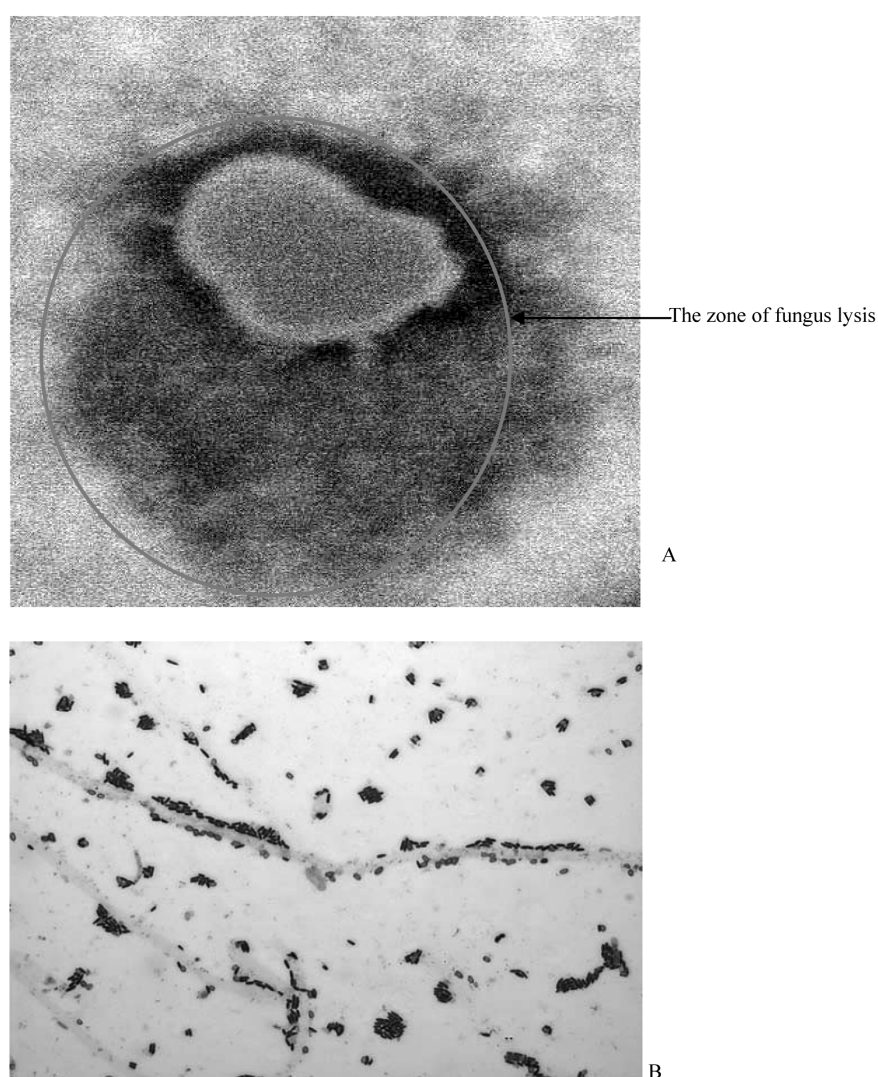


Fig. 2. Antagonistic properties of sporulating bacteria of *Bacillus* genus on peptone-agar medium against pathogenic fungi from *Fusarium* genus. A – macroscopic image; B – microscopic image



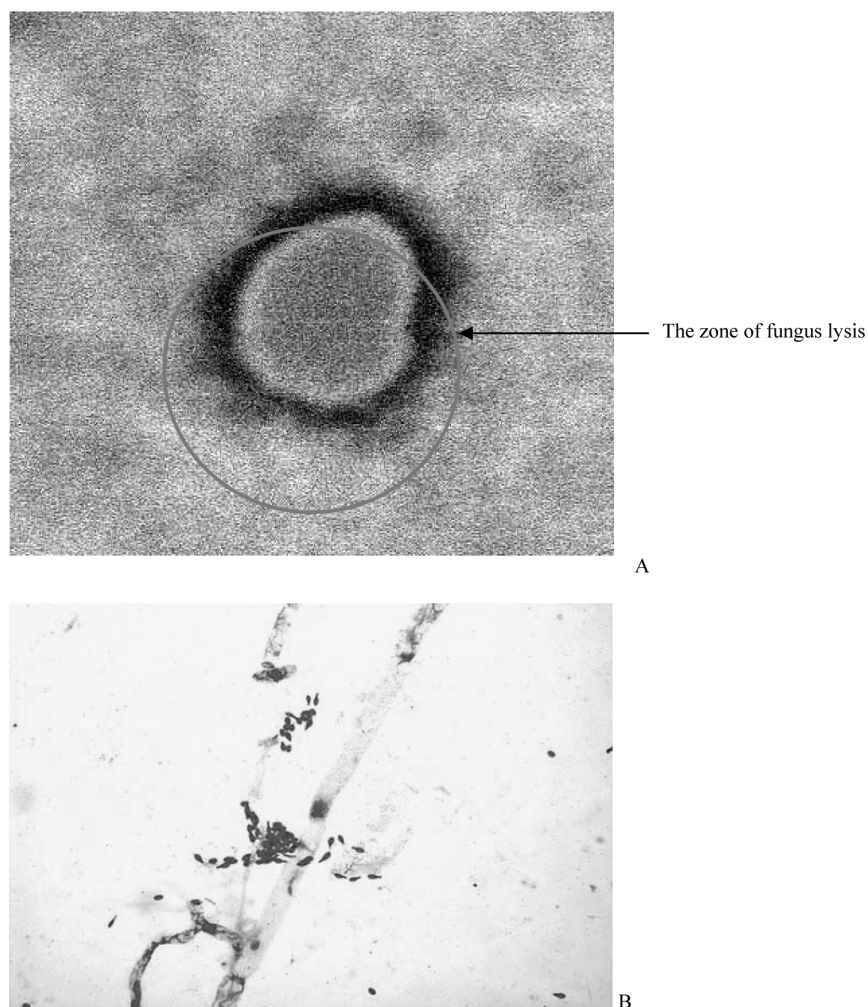


Fig. 3. Antagonistic properties of sporulating bacteria of *Clostridium* genus on peptone-agar medium against pathogenic fungi from *Fusarium* genus. A – macroscopic image; B – microscopic image

On the other hand, bacteria from *Bacillus* and *Pseudomonas* genera were found to exert a stimulating effect in relation to the examined fungi on the PDA medium which could be attributed to the secretion by those bacteria of vitamins from group B as well as of IAA hormone. Diby et al. [2005] claims that the secretion of the vitamin and indoleacetic acid by bacteria can be attributed to the influence of the medium as its composition, on the one hand, stimulates production of the above-mentioned substances and, on the other, additionally the medium itself stimulates growth of the examined microorganisms.

## CONCLUSIONS

1. Strains of sporulating bacteria from *Bacillus* and *Clostridium* genera as well as fluorescent bacteria from the *Pseudomonas* genus isolated from the rhizosphere zone in “*in vitro*” conditions were characterised by strong antagonism against cereal phytopathogens from the *Fusarium* genus only on the applied peptone-agar medium.
2. On the PDA medium, bacteria from the *Bacillus* and *Pseudomonas* genera exhibited a positive taxis against fungal pathogens from the *Fusarium* genus, whereas bacteria from the *Clostridium* genus turned out to be neutral.

## REFERENCES

- Benizri E., Courtade A., Guckert A. 1995. Fate of two microorganisms in maize simulated rhizosphere under hydroponic and sterile conditions. *Soil Biol. Biochem.* 27: 71–77.
- Diby P., Saju K.A., Jisha P.J., Sarma Y.R., Kumar A., Anandaraj M. 2005. Mycolytic enzymes produced by *Pseudomonas fluorescens* and *Trichoderma* spp. against *Phytophthora capsici*, the foot rot pathogen of black pepper (*Piper nigrum*). *Ann. Microbiol.* 55: 129–133.
- Fuchs R., Schäfer M., Geoffroy V., Meyer J.M. 2001. Siderotyping – a powerful tool for the characterization of pyoverdines. *Curr. Top. Med. Chem.* 1: 31–57.
- Handelsman J., Stabb E.V. 1996. Bicontrol of soilborne plant pathogens. *Plant Cell.* 8: 1855–1869.
- Jankiewicz U. 2010. Bioactive metabolites of rhizosphere bacteria *Pseudomonas*. *Woda Środ. Obsz. Wiek-skie.* 10(2): 83–92.
- Jankiewicz U., Kuzawińska O. 2009. Purification and partial characterization of pyoverdine synthesized by *Pseudomonas putida*. *EJPAU*, 12(1), #11.
- Kunicki-Goldfinger W.J.H. 2001. *Life of bacteria*. PWN Warszawa: ss. 615.
- Nagarajkumar M., Bhaskaran R., Velazhahan R. 2004. Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. *Microbiol. Res.* 159: 73–78.
- Niewiadomska A., Sawińska Z., Wolna-Maruwka A. 2012. Impact of seed dressing on microbiological activity of soil under winter triticale cultivation. *Archiv. Environ. Protec.* 38(2): 93–105.
- Ševčuk I.V., Gorevoj L.F., Tesljuk V.V. 2004. Bifungicid Misan – Vot boleznej jabloni. W: *Nateriali mižnarodnoji naukovo-praktičnoji konferenciji “Integrovanij zachist roslin na počatku XXI stolittja”*. Kiiv: 502–504.
- Sharma S.K. 2002. Control of white root rot of apple caused by *Dematophora necatrix* with *Bacillus* sp. *Plant Dis. Res.* 17: 308–312
- Škvarčuk J.M. 2000. Rol’ „Oblbiopidprijemstva”v integrovanomu zchisti sil’gospkul’tur v Zakarpatti. W: *Integrovanij zachist plodovich kul’tur i vinogradu*. Ugorom: 136–138.
- Zydlik P. 2008. Wykorzystanie preparatów pochodzenia naturalnego w zwalczaniu niektórych chorób roślin sadowniczych. *Nauka Przyr. Technol.* 2(1), #3.

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**ANTAGONIZM PRZETRWAŁNIKUJĄCYCH BAKTERII *BACILLUS* I *CLOSTRIDIUM*  
ORAZ FLUORESCENCYJNYCH *PSEUDOMONAS* WYIZOLOWANYCH SPOD KUKURYDZY  
Z PATOGENICZNYMI GATUNKAMI *FUSARIUM*, NA RÓŻNYCH PODŁOŻACH  
HODOWLANYCH**

**Synopsis.** Istnieją liczne doniesienia o produkcji przez bakterie w warunkach *in vitro* przeciwgrzybowych i przeciwbakteryjnych metabolitów, które mogą działać też *in vivo*. Wytwarzane przez mikroorganizmy substancje mają zdolność antagonistycznego oddziaływania na inne drobnoustroje oraz indukowania systemicznej odporności roślin. Antagonizm obejmuje trzy rodzaje interakcji między organizmami: amensalizm, współzawodnictwo o miejsce i pokarm oraz pasożytnictwo. W oparciu o dotychczasowy stan wiedzy i wyniki badań coraz częściej wykorzystuje się te zdolności bakterii w biologicznej ochronie roślin. Celem badań było wyizolowanie bakterii o właściwościach antygrzybowych z gleby spod uprawy kukurydzy i zbadanie ich właściwości antagonistycznych w stosunku do trzech gatunków grzybów: *Fusarium cullmorum*, *Fusarium oxysporum* i *Fusarium poae* na dwóch różnych podłożach hodowlanych: glukozowo ziemniaczanym (ang. PDA) i peptonowo agarowym. Bakterie o właściwościach antygrzybowych izolowane były z gleby spod uprawy kukurydzy, na pożywce King B. Aktywność mikolityczną izolowanych szczepów badano na różnych podłożach. Patogeniczne szczepy grzybów otrzymano z Katedry Fitopatologii, Uniwersytetu Przyrodniczego w Poznaniu. Otrzymane wyniki wskazują na to, że właściwości mikolityczne wyizolowanych szczepów zależne były od rodzaju zastosowanego podłoża. Wrażliwość grzybów wobec wyizolowanych rodzajów bakterii notowano zawsze na pożywce peptonowo-agarowej, w przeciwieństwie do pożywki PDA, na której takich zależności nie obserwowano

**Słowa kluczowe:** mykoliza, *Fusarium*, *Pseudomonas*, *Bacillus*, *Clostridium*, pożywka glukozowo-ziemniaczana, pożywka peptonowo-agarowa

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