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ANTAGONISTIC ACTIVITY OF INDIGENOUS *PSEUDOMONAS* ISOLATES AGAINST *FUSARIUM* SPECIES ISOLATED FROM ANISE

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Abstract

Fusarium species are widely distributed and responsible for several plant diseases in different medicinal plants. Fungi of this genera cause very important economic losses in Serbian plantation. Antibiotic production by plant-associated microorganisms represents an environmentally acceptable method of disease control, esspecialy in cultivation of medicinal and aromatic plants. Among the plant growth promoting bacteria (PGPB), Pseudomonas have been recognized as the most frequent antagonists of plant fungal pathogens and antibiotic producers. This is probably due to the widely distribution of this diverse group of bacteria in temperate soils and their often predomination among bacteria from plant rhizosphere. In this study, we examined the antifungal activity of eleven indigenous Pseudomonas isolates (PB4, PB5, K38, Q34, PBA12, PD5, C7, C8, Q16P, K29 and K35) against eight phytopathogenic fungi belonging to genus Fusarium (Fusarium tricinctum, F. sambucinum, F. equiseti, F. heterosporum, F. sporotrichioides, F. semitectum, F. verticillioides and F. oxysporum), which had infected anise (Pimpinella anisum L., fam. Apiaceae), using in vitro growth inhibition tests. The obtained results demonstrated that all Pseudomanas isolates showed more or less pronounced antifungal activity, whereby the most pronounced activity was observed for K29 and K35 strains. F. oxysporum and F. verticillioides showed the highest sensitivity to antibiotic-producing Pseudomanas isolates. In general, it has been concluded that studied Pseudomonas isolates have potential in controlling plant diseases caused by Fusarium spp., whereby the bacterial isolates with the highest inhibitory potential will be selected for further experiments.

Key words*: Pseudomonas, Fusarium spp., Pimpinella anisum, antifungal activity.*

INTRODUCTION

The use of chemical fertilizers and pesticides has caused an incredible harm to the environment. These agents are both hazardous to animals and humans and may persist and accumulate in natural ecosystems. An answer to this problem is replacing chemicals with biological approaches, which are considered more environment friendly in the long term. One of the emerging research area for the control of different phytopathogenic agents is the use of biocontrol plant growth promoting rhizobacteria (PGPR), which are capable of suppressing or preventing the phytopathogen damage (Nihorembere et al., 2011).

Phytopathogenic fungi, as the most common plant pathogens, are capable of infecting different types of plant tissues. Among the main aims in agriculture is finding adequate strategies for their suppression. One of these strategies is biological control (biocontrol) of plant diseases that relies on the use of natural antagonists of phytopathogenic fungi (Heydari and Pessarakli, 2010).

A special place among the natural antagonists of phytopathogenic fungi belongs to rhizobacteria that show beneficial effects on plant growth (PGPR) (Zehnder et al., 2001). These bacteria use various mechanisms for their action: production of plant hormones, asymbiotic fixation of N_2, antagonism towards phytopathogenic microorganisms and the ability to solubilize mineral phosphates and other nutrients (Cattelan et al., 1999). Different isolates of fluorescent *Pseudomonas* species take prominent place in this respect.

Consequently, these isolates have been intensively studied.

Fluorescent *Pseudomonas* species are present in temperate and tropical soils, often dominant among rhizobacteria (Ayyadurai et al., 2007). They belong to PGPR because of the ability to colonize the roots of plants and stimulate growth by decreasing the frequency of diseases. Suppression of diseases includes the inhibition of pathogens by competition and/or by antagonism (Couillerot et al., 2009). The prominent feature of fluorescent *Pseudomonas* species is the production of antibiotics as inhibitory compounds that play a role in the suppression of diseases caused by phytopathogenic fungi (Haas and Défago, 2005). One of the best-studied antibiotics of fluorescent *Pseudomonas* species are phenazines, nitrogen-containing heterocyclic compounds (Fernando et al., 2005). The only known natural producers of phenazines are bacteria (Pierson III and Pierson, 2010).

Fluorescent *Pseudomonas* species are capable of inhibiting the phytopathogenic fungi that belong to genus *Fusarium* (Showkat et al., 2012). *Fusarium* spp. are a widespread cosmopolitan group of fungi and commonly colonize aerial and subterranean plant parts, either as primary or secondary invaders. Some species are common in soil and it is rare to find necrotic root of a plant in most agricultural soils that is not colonized by at least one *Fusarium* sp. (Nelson et al., 1983).

One of the hosts of *Fusarium* spp. is anise (*Pimpinella anisum* L., fam. Apiaceae). Anise is an aromatic plant which is used in traditional medicine (especially its fruits) as carminative, aromatic, disinfectant and galactagogue (Shojaii and Abdollahi Fard, 2012).

The aim of this study was to examine the antifungal activity of eleven indigenous *Pseudomonas* isolates against the eight phytopathogenic fungi belonging to genus *Fusarium*, which had infected anise (*Pimpinella anisum* L., fam. Apiaceae).

MATERIALS AND METHODS

The antifungal activity of the following indigenous *Pseudomonas* isolates: PB4, PB5, K38, Q34, PBA12, PD5, C7, C8, Q16P, K29 and K35, was examined against the phytopathogenic fungi belonging to genus *Fusarium* (*F. oxysporum*, *F. tricinctum*, *F. sambucinum*, *F. equiseti*, *F. heterosporum*, *F. sporotrichioides*, *F. semitectum*, *F. verticillioides*), which had infected anise (*Pimpinella anisum* L., fam. Apiaceae).

The examination was conducted on Waksman agar plates nutrient media, using *in vitro* inhibition tests. Overnight cultures of the tested *Pseudomonas* isolates, optimized to $1 \cdot 10^7$ cfu/ml were used to examine the influence of extracellular metabolites of cells (1 ml of cultures was centrifuged at 13000 rpm for 10 min and resuspended in the same volume of sterile saline solution).

Inoculation of Waksman nutrient media with the tested cultures of *Pseudomonas* isolates was done near the edges of Petri dishes and mycelia of the studied *Fusarium* species were placed in the center. Control variants contained only mycelia of *Fusarium* species on Waksman agar plates.

Observation and the measuring of zones of growth inhibition of mycelia around bacterial colonies were performed after seven days of incubation at 25°C (Nair and Anith, 2009). The percentage of growth inhibition of mycelia of *Fusarium* species was calculated by the formula: % Inhibition = [(Control - Treatment)/Control] x 100 (Ogbebor and Adekunle, 2005).

RESULTS AND DISCUSSIONS

Due to the soil-borne nature of the diseases caused by *Fusarium* species the use of chemical methods for the control of disease is rarely successful. Inconsistencies in biocontrol under varying environmental conditions have been a common limitation of soil-borne pathogens. The present research was conducted to evaluate the efficacy of indigenous *Pseudomonas* isolates against these pathogens.

Table 1 displays the data on *in vitro* antifungal activity of selected *Pseudomonas* sp. isolates toward *Fusarium* species, which had infected anise.

The obtained results imposed that all *Pseudomonas* isolates showed more or less pronounced antifungal activity, whereby the mycelial growth of *Fusarium* species was inhibited in the range of 3.33% (for

Pseudomonas isolates PB4 and PB5 toward *F. tricinctum*) to 77.78% (for *Pseudomonas* isolates K29 and K35 toward *F. oxysporum*).
The highest percentage of growth inhibition was caused by *Pseudomonas* isolates K29 (from 35.71% toward *F. equiseti* to 77.78% toward *F. oxysporum*) and K35 (from 37.50% toward *F. semitectum* to 77.78% toward *F. oxysporum*).
The lowest percentage of inhibition was caused by the following *Pseudomonas* isolates: PB4 (from 3.33% toward *F. tricinctum* to 48.89% toward *F. oxysporum*), PB5 (from 3.33% toward *F. tricinctum* to 51.11% toward *F. oxysporum*), PBA12 (from 13.33% toward *F. tricinctum* to 51.11% toward *F. verticillioides*), PD5 (from 13.33% toward *F. tricinctum* to 53.33% toward *F. verticillioides*).
In general, *F. oxysporum* and *F. verticillioides* showed the highest sensitivity to antibiotic-producing *Pseudomanas* isolates.
Antifungal activity of indigenous *Pseudomonas* isolates was also confirmed in other investigation (Jošić et al., 2012). In addition, *in vitro* assays in previous studies (Velusamy et al., 2011; Shojaii and Abdollahi Fard, 2012) revealed high sensitivity of *F. oxysporum* to *Pseudomonas* sp. as in the present research. As pronounced by other authors (Karimi et al., 2012), PGPR can be used in the biocontrol of phytopathogens.

Table 1. Antifungal activity of selected *Pseudomonas* sp. isolates toward *Fusarium* species (F1 - *Fusarium tricinctum*; F2 - *F. sambucinum*; F3 - *F. equiseti*; F4 - *F. heterosporum*; F5 - *F. sporotrichioides*; F6 - *F. semitectum*; F7 - *F. verticillioides*; F8 - *F. oxysporum*)

Pseudomonas sp. isolates	*Fusarium* species							
	F1	F2	F3	F4	F5	F6	F7	F8
PB4	3.33*	25.71	21.43	20.00	35.56	18.75	42.22	48.89
PB5	3.33	25.71	28.57	8.00	40.00	37.50	42.22	51.11
K38	23.33	17.14	28.57	24.00	33.33	43.75	55.56	44.44
Q34	16.67	17.14	21.43	24.00	33.33	43.75	51.11	51.11
PBA12	13.33	14.29	28.57	8.00	22.22	37.50	51.11	48.89
PD5	13.33	5.71	21.43	12.00	22.22	43.75	53.33	46.67
C7	43.33	34.29	21.43	24.00	44.44	12.50	57.78	51.11
C8	36.67	28.57	21.43	20.00	44.44	25.00	53.33	51.11
Q16P	56.67	42.86	35.71	40.00	66.67	37.50	60.00	71.11
K29	66.67	54.29	35.71	44.00	64.44	43.75	66.67	77.78
K35	60.00	54.29	42.86	40.00	64.44	37.50	66.67	77.78

*Inhibition (in %)

CONCLUSIONS

Biological control of *Fusarium* species, one of the most aggressive isolates from medicinal plants in Serbia, isolated from anise, is an ecological method of plant protection.
Our investigation confirmed more or less pronounced antifungal activity of all tested *Pseudomonas* isolates, whereby the most pronounced activity was observed for K29 and K35 strains. Regarding the *Fusarium* species, the highest sensitivity to antibiotic-producing *Pseudomanas* isolates was observed for *F. oxysporum* and *F. verticillioides*.
Our findings impose that the studied *Pseudomonas* isolates have potential in controlling plant diseases caused by *Fusarium* spp., whereby the bacterial isolates with the highest inhibitory potential will be selected for further experiments.

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