Original scientific paper
10.7251/AGSY1404582S

INDIGENOUS PSEUDOMONAS CHLORORAPHIS AFFECTS GROWTH OF ALTERNARIA SP., PHOMA SP. AND DRECHSLERA TETRAMERA FROM ANISE

Aleksandra STANOJKOVIC SEBIC[1*], Snezana PAVLOVIC[2], Mira STAROVIC[2], Radmila PIVIC[1], Dobrivoj POSTIC[2], Zorica LEPSANOVIC[3], Dragana JOSIC[1]

[1]Institute of Soil Science, Belgrade, Serbia
[2]Institute for Medicinal Plant Research "Dr. J. Pančić", Belgrade, Serbia
[3]Military Medical Academy, Belgrade, Serbia
*Corresponding author: astanojkovic@yahoo.com

Abstract

Pseudomonas chlororaphis is a bacterium used as a soil inoculant in agriculture and horticulture. It can act as a biocontrol agent against certain phytopathogenic fungi via production of phenazine type antibiotics. Phytopathogenic fungi are responsible for several plant diseases in different medicinal plants and cause very important economic losses in Serbian plantation. Among them, *Alternaria* sp., *Phoma* sp. and *Drechslera tetramera* are known as major plant pathogens, whereby at least 20% of agricultural spoilage is caused by *Alternaria* species. In this study, we examined the antifungal activity of indigenous *Pseudomonas chlororaphis* isolates against the phytopathogenic fungi *Alternaria* sp., *Phoma* sp. and *Drechslera tetramera*, which had infected anise (*Pimpinella anisum* L., fam. Apiaceae), using *in vitro* inhibition tests. The obtained results showed that studied antibiotic-producing *Ps. chlororaphis* isolates inhibited the tested fungi as follows: *D. tetramera* in the range from 3.85-69.23%, *Alternaria* sp. in the range from 5-50%, and *Phoma* sp. in the range from 0-50%. In general, it has been concluded that studied *P. chlororaphis* isolates have potential in controlling plant diseases caused by phytopathogenic fungi *Alternaria* sp., *Phoma* sp. and *D. tetramera*, whereby the bacterial isolates with the highest inhibitory potential will be selected for further experiments.

Key words: *Pseudomonas chlororaphis isolates, phytopathogenic fungi, Pimpinella anisum, antifungal activity.*

Introduction

Pathogenic microorganisms affecting plant health are a major and chronic threat to food production and ecosystem stability worldwide. As agricultural production intensified over the past few decades, producers became more and more dependent on agrochemicals as a relatively reliable method of crop protection helping with economic stability of their operations. However, increasing use of chemical inputs causes several negative effects, i.e., development of pathogen resistance to the applied agents and their non-target environmental impacts (Compant et al., 2005). 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 areas for the control of different phytopathogenic agents is the use of biocontrol activities of microorganisms, which are capable of suppressing or preventing the damages caused by phytopathogens (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 N2, 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. 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, nitrogencontaining heterocyclic compounds (Fernando et al., 2005). The only known natural producers of phenazines are bacteria (Pierson III and Pierson, 2010). Among fluorescent *Pseudomonas* species a bacterium *Pseudomonas chlororaphis* is used as a soil inoculant in agriculture and horticulture. It can act as a biocontrol agent against certain phytopathogenic fungi via production of phenazine type antibiotics (Maddula et al., 2008; Shen et al., 2012).
Phytopathogenic fungi are responsible for several plant diseases in different medicinal plants and cause very important economic losses in Serbian plantation. Among them, *Alternaria* sp., *Phoma* sp. and *Drechslera tetramera* are known as major plant pathogens, whereby at least 20% of agricultural spoilage is caused by *Alternaria* species. The genus *Alternaria* is widely distributed in nature and its species are among the most common fungi on the phyllosphere (Lopes and Martins, 2008). It includes both plant-pathogenic and plant-saprophytic species that may damage crops in the field or cause post-harvest decay (Griffin and Chu, 1983), causing considerable economic losses for farmers and food industries. In addition, the genus produces mycotoxins and phytotoxins. The toxins alternariol, alternariol methyl ether, altenuene, and tenuazonic acid are known as possible food contaminants with potential toxicological risk (Pose et al., 2004). Most *Phoma* species are saprobes, but have plant pathogenic potential causing numerous diseases of vegetables and other annual plants (Schwartz and Mohan, 1999; Boerema et al., 2004). *Phoma* sp. infects subterranean organs directly and aerial parts of the plants indirectly (Boerema et al., 2004). The species *Drechslera tetramera* was first described causing foot rot in winter wheat (McKinney, 1925). It was reported to cause crown and root rot disease, leaf spot (Singh and Lal, 1965; Naphade, 1968) and storage disease in many plants (Rao, 1967). The species was also observed on seeds of many crops (Chidambaram et al., 1973). One of the hosts of *Alternaria* sp., *Phoma* sp. and *Drechslera tetramera* 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). Regarding the widely distribution and phytopathogenicity of *Alternaria* sp., *Phoma* sp. and *Drechslera tetramera*, as well as the capability of *Pseudomonas chlororaphis* isolates to inhibit the phytopathogenic fungi, the aim of this study was to examine the antifungal activity of thirteen indigenous *P. chlororaphis* isolates against the phytopathogenic fungi *Alternaria* sp., *Phoma* sp. and *Drechslera tetramera*, which had infected anise (*Pimpinella anisum* L., fam. Apiaceae).

Material and methods

The antifungal activity of the following indigenous *Pseudomonas chlororaphis* isolates: PB4, PB5, K38, Q34, M28, B25, PBA12, PD5, C7, C8, Q16P, K24, K29 and K35, was examined

against the phytopathogenic fungi *Alternaria* sp., *Phoma* sp. and *Drechslera tetramera*, which had infected anise (*Pimpinella anisum* L., fam. Apiaceae).
The study was carried out in the Genetics Section of the Institute of Soil Science, Belgrade, from February to April in 2014. The examination was conducted on Waksman agar nutrient media, using *in vitro* inhibition tests. Overnight cultures of the tested *P. chlororaphis* 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).
The sowing of Waksman nutrient media with the tested cultures of *P. chlororaphis* isolates was done near the edges of Petri dishes and mycelia of the studied phytopathogenic fungi were placed in the center. Control variants contained only mycelia of phytopathogenic fungi on Waksman agar plates.
Observation and the measuring of zones of mycelia growth inhibition 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 *Alternaria* sp., *Phoma* sp. and *D. tetramera* was calculated by the formula: % Inhibition = [(Control - Treatment)/Control] x 100 (Ogbebor and Adekunle, 2005).

Results and discussion

Due to the soil-borne nature of the diseases caused by *Alternaria* sp., *Phoma* sp. and *D. tetramera* 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 *P. chlororaphis* isolates against these pathogens.
Table 1 displays the data on *in vitro* antifungal activity of selected *P. chlororaphis* isolates toward *Alternaria* sp., *Phoma* sp. and *D. tetramera*, which had infected anise.
The obtained results imposed that all studied antibiotic-producing *P. chlororaphis* isolates showed more or less pronounced antifungal activity and inhibited the tested fungi as follows: *D. tetramera* in the range from 3.85-69.23%, *Alternaria* sp. in the range from 5-50%, and *Phoma* sp. in the range from 0-50%.
The highest percentage of growth inhibition was caused by the following *P. chlororaphis* isolates: K24 (from 50.00% toward *Phoma* sp. and *Alternaria* sp. to 69.23% toward *D. tetramera*), K35 (from 40.00% toward *Alternaria* sp. to 69.23% toward *D. tetramera*), K29 (from 40.00% toward *Alternaria* sp. to 65.38% toward *D. tetramera*), Q16P (from 40.00% toward *Alternaria* sp. to 69.23% toward *D. tetramera*) and M28 (from 35.00% toward *Alternaria* sp. to 61.54% toward *D. tetramera*).
The lowest percentage of inhibition was caused by *P. chlororaphis* isolates PD5 (from 0.00% toward *Phoma* sp. to 15.00% toward *Alternaria* sp.) and PBA12 (from 0.00% toward *Phoma* sp. to 30.00% toward *Alternaria* sp.).
In general, *D. tetramera* showed the highest sensitivity to antibiotic-producing *P. chlororaphis* isolates.
Figures 1 and 2 display the zones of inhibition, caused by the most active *P. chlororaphis* isolates toward *Phoma* sp. and *D. tetramera*.
More or less pronounced antifungal activity of indigenous *Pseudomonas* isolates toward phytopathogenic fungi *Alternaria* sp. and *D. tetramera* was also confirmed in other investigations (Jošić et al., 2012; 2012a; 2012b). In addition, *in vitro* assays in previous studies (Lovic et al., 1993; Avinash and Ravishankar Rai, 2014) revealed that PGPR strains showed good antifungal activity against *Phoma* sp. As pronounced by other authors (Haas and Défago, 2005; Couillerot et al., 2009; Karimi et al., 2012), PGPR can be used in the biocontrol of phytopathogens.

Table 1. Antifungal activity of selected *Pseudomonas chlororaphis* isolates toward phytopathogenic fungi *Alternaria* sp., *Phoma* sp. and *Drechslera tetramera*

Pseudomonas chlororaphis isolates	*Alternaria* sp.	*Phoma* sp.	*Drechslera tetramera*
PB4	10.00*	10.00	26.92
PB5	10.00	15.00	30.77
K38	20.00	10.00	23.08
Q34	30.00	15.00	26.92
M28	35.00	45.00	61.54
B25	15.00	10.00	26.92
PBA12	30.00	0.00	11.54
PD5	15.00	0.00	3.85
C7	15.00	20.00	61.54
C8	5.00	20.00	61.54
Q16P	40.00	45.00	69.23
K24	40.00	50.00	69.23
K29	40.00	50.00	65.38
K35	50.00	50.00	69.23

*Inhibition (in %).

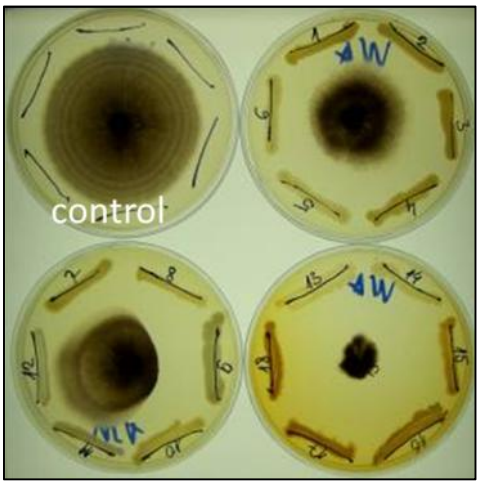

Figure 1. Zones of inhibition caused by *P. chlororaphis* isolates toward *Drechslera tetramera*

Figure 2. Zones of inhibition caused by *P. chlororaphis* isolates toward *Phoma* sp.

Conclusion

Biological control of phytopathogenic fungi *Alternaria* sp., *Phoma* sp. and *D. tetramera*, causing considerable economic losses in cultivation of medicinal plants in Serbia, is an ecological method of plant protection. In this regard, different isolates of fluorescent *P. chlororaphis* species have been intensively studied.

Our investigation confirmed more or less pronounced antifungal activity of all tested *P. chlororaphis* isolates, whereby the most pronounced activity was observed for K24, K35, K29, Q16P and M28 strains. Regarding the studied phytopathogenic fungi, the highest sensitivity to antibiotic-producing *P. chlororaphis* isolates was observed for *D. tetramera*.

Our findings impose that the studied *P. chlororaphis* isolates have potential in controlling plant diseases caused by phytopathogenic fungi *Alternaria* sp., *Phoma* sp. and *D. tetramera*, whereby the bacterial isolates with the highest inhibitory potential will be selected for further experiments.

Acknowledgements

This research was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Project III46007.

References

Avinash, TS, Ravishankar Rai, V, (2014). Antifungal activity of plant growth promoting rhizobacteria against Fusarium oxysporum and Phoma sp. of Cucurbitaceae, In: Kharwar, RN, Upadhyay, R, Dubey, N, Raghuwanshi, R. (eds), Microbial Diversity and Biotechnology in Food Security, Springer, India, 257-264.

Ayyadurai, N, Ravindra Naik, P, Sakthivel, N, (2007). Functional characterization of antagonistic fluorescent pseudomonads associated with rhizospheric soil of rice (Oryza sativa L.), Journal of Microbiology and Biotechnology, 17, 919-927.

Boerema, GH, de Gruyter, J, Noordeloos, ME, Hamers, MEC, (2004). Phoma Identification Manual: Differentiation of Specific and Infra-specific Taxa in Culture, CABI Publishing, Wallingford, Oxfordshire, UK.

Cattelan, AJ, Hartel PG, Fuhrmann, JJ, (1999). Screening for plant growth-promoting rhizobacteria to promote early soybean growth, Soil Science Society of America Journal, 63, 1670-1680.

Chidambaramp, P, Mathur, SB, Neergaard, P, (1973). Identification of seed-borne Drechslera species, Friesia, X (3), 165-208.

Couillerot, O, Prigent-Combaret, C, Caballero-Mellado, J, Moënne-Loccoz, Y, (2009). Pseudomonas fluorescens and closely related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens, Letters in Applied Microbiology, 48, 505-512.

Fernando, WGD, Nakkeeran, S, Zhang, Y, (2005). Biosynthesis of antibiotics by PGPR and its relation in biocontrol of plant diseases, In: Siddiqui, ZA (ed), PGPR: Biocontrol and Biofertilization, Springer Science, Dordrecht, The Netherlands, 67-109.

Griffin, GF, Chu, FS, (1983). Toxicity of the Alternaria metabolites alternariol, alternariol methyl ether, altenuene, and tenuazonic acid in the chicken embryo assay, Applied and Environmental Microbiology, 46, 1420-1422.

Haas, D, Défago, G, (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads, Nature Reviews Microbiology, 3, 307-319.

Heydari, A, Pessarakli, M, (2010). A review on biological control of fungal plant pathogens using microbial antagonists, Journal of Biological Sciences, 10, 273-290.

Jošić, D, Protolipac, K, Starović, M, Stojanović, S, Pavlović, S, Miladinović, M, Radović, S, (2012). Phenazines producing Pseudomonas isolates decrease Alternaria tenuissima growth, pathogenicity and disease incidence on cardoon, Archives of Biological Sciences, Belgrade, 64, 1495-1503.

Jošić, D, Pivić, R, Miladinović, M, Starović, M, Pavlović, S, Đurić, S, Jarak, M, (2012a). Antifungal activity and genetic diversity of selected Pseudomonas spp. from maize rhizosphere in Vojvodina, Genetika, 44 (2), 377-388.

Jošić, D, Delić, D, Rasulić, N, Stajković, O, Kuzmanović, Đ, Stanojković, A, Pivić, R, (2012b). Indigenous Pseudomonads from rhizosphere of maize grown on pseudogley soil in Serbia, Bulgarian Journal of Agricultural Science, 18, 197-206

Karimi, K, Amini, J, Harighi, B, Bahramnejad, B, (2012). Evaluation of biocontrol potential of Pseudomonas and Bacillus spp. against Fusarium wilt of chickpea, Australian Journal of Crop Science, 6, 695-703.

Lopes, MC, Martins, VC, (2008). Fungal plant pathogens in Portugal: Alternaria dauci, Revista Iberoamericana de Micología, 25, 254-256.

Lovic, B, Heck, C, Gallian, JJ, Anderson, AJ, (1993). Inhibition of the sugarbeet pathogens Phoma betae and Rhizoctonia solani by bacteria associated with sugarbeet seeds and roots, Journal of Sugar Beet Research, 30 (31), 169-184.

Maddula, VSRK, Pierson, EA, Pierson III, LS, (2008). Altering the ratio of phenazines in Pseudomonas chlororaphis (aureofaciens) strain 30-84: effects on biofilm formation and pathogen inhibition. Journal of Bacteriology, 190, 2759-2766.

McKinney, HH, (1925). Foot-rot diseases of wheat in America, Bulletin of the U.S. Department of Agriculture, 134 (7), 33.

Nair, CB, Anith, KN, (2009). Efficacy of acibenzolar-smethyl and rhizobacteria for the management of foliar blight disease of amaranth, Journal of Tropical Agriculture, 47, 43-47.

Naphade, SR, (1968). A new leaf blight disease of Crotalaria juncea from India, Plant Disease Reporter, 52, 377-378.

Nihorembere, V, Ongena, M, Smargiass, M, (2011). Beneficial effect of the rhizosphere microbial community for plant growth and health, Biotechnologie, Agronomie, Société et Environnement, 15, 327-337.

Ogbebor, N, Adekunle, AT, (2005). Inhibition of conidial germination and mycelial growth of Corynespora cassiicola (Berk and Curt) of rubber (Hevea brasiliensis muell. Arg.) using extracts of some plants, African Jornal of Biotechnology, 4, 996-1000.

Pierson III, LS, Pierson, EA, (2010). Metabolism and function of phenazines in bacteria: impacts on the behavior of bacteria in the environment and biotechnological processes, Applied Microbiology and Biotechnology, 86, 1659-1670.

Pose, G, Ludemann,V, Segura J, Fernández Pinto, V, (2004). Mycotoxin production by Alternaria strains isolated from tomatoes affected by Blackmold in Argentina, Mycotoxin Research, 20, 80-86.

Schwartz, HF, Mohan, SK, (1999). Compendium of Onion and Garlic Diseases, The American Phytopathological Society Press, St. Paul, MN.

Shen, X, Chen, M, Hu, H, Wang, W, Peng, H, Xu, P, Zhang, X, (2012). Genome sequence of Pseudomonas chlororaphis GP72, a root-colonizing biocontrol strain, Journal of Bacteriology, 194 (5), 1269-1270.

Shojaii, A, Abdollahi Fard, M, (2012). Review of pharmacological properties and chemical constituents of Pimpinella anisum, ISRN Pharmaceutics, 510795.

Singh, GP, Lal, S, (1965). A new leaf spot disease of Singhara (Trapa bispinosa) caused by Bipolaris tetramera, Indian Phytopathology, 18, 85-87.

Zehnder, GW, Murphy, JF, Sikora, EJ, Kloepper, JW, (2001). Application of rhizobacteria for induced resistance, European Journal of Plant Pathology, 107, 39-50.