Biblid: 1821-4487 (2020) 24; 2; p 72-76
UDK: 582.926.2:632.35
DOI: 10.5937/jpea24-27076

Original Scientific Paper
Originalni naučni rad

IN VITRO POTENTIAL OF BACILLUS SPP. ANTAGONISTS FOR SUPPRESSION OF XANTHOMONAS EUVESICATORIA PHYTOPATHOGENS

IN VITRO POTENCIJAL ANTAGONISTA BACILLUS SPP. ZA SUZBIJANJE FITOPATOGENA XANTHOMONAS EUVESICATORIA

Ivana PAJČIN[*], Vanja VLAJKOV[*], Zorana RONČEVIĆ[*], Jelena DODIĆ[*],
Mila GRAHOVAC[**], Maja IGNJATOV[***], Jovana GRAHOVAC[*]
[*]University of Novi Sad, Faculty of Technology Novi Sad, 21000 Novi Sad, Bulevar cara Lazara 1, Serbia
[**] University of Novi Sad, Faculty of Agriculture, 21000 Novi Sad, Trg Dositeja Obradovića 8, Serbia
[***]Institute of Field and Vegetable Crops, 21000 Novi Sad, Maksima Gorkog 30, Serbia
e-mail:paj@tf.uns.ac.rs

ABSTRACT

Bacterial pathogen Xanthomonas euvesicatoria, principal causer of bacterial spot, represents a significant problem in agricultural practice due to high yield losses in the production of pepper and tomato. The development of resistance to copper pesticides has shifted research, in the field of its suppression, towards biopesticides. In this study, several Bacillus strains were tested against Xanthomonas euvesicatoria strains, isolated from pepper leaves with symptoms of bacterial spot, to select a sufficiently effective antagonist. When it comes to the testing of cultivation broth, containing biomass of tested antagonists, the best results were achieved using isolate Bacillus sp3. On the other hand, when biomass-free supernatants, containing produced antimicrobial compounds, were tested, Bacillus sp1 and Bacillus sp2 have shown the highest antimicrobial activity. The results of this study represent a basis for further development of bioprocess solutions for the production of biopesticides based on Bacillus spp. biomass or antimicrobial compounds, showing high efficiency in suppression of pepper bacterial spot.

***Keywords**: antimicrobial activity, pepper, bacterial spot, biomass, antimicrobial compounds, sensitivity.*

REZIME

Bakterijski patogen Xanthomonas euvesicatoria predstavlja glavnog uzročnika bakteriozne pegavosti, stoga predstavlja značajan problem u poljoprivrednoj praksi usled izazivanja velikih gubitaka prinosa u proizvodnji paprike i paradajza. Uobičajena sredstva za suzbijanje i kontrolu ovog fitopatogena predstavljaju preparati na bazi bakra. Međutim, razvoj rezistentnosti prema pesticidima na bazi bakra doveo je do zaokreta u polju suzbijanja ovih patogena ka primeni biopesticida, odnosno bioloških kontrolnih agensa. Bakterije roda Bacillus i njihovi metaboliti sa izraženom antimikrobnom aktivnošću protiv ciljanih fitopatogena predstavljaju najperspektivnije aktivne komponente biokontrolnih preparata za zaštitu bilja. U ovom istraživanju nekoliko sojeva roda Bacillus ispitano je sa ciljem odabira antagonista dovoljno efikasnih u suzbijanju sojeva Xanthomonas euvesicatoria, koji su izolovani sa listova paprike sa simptomima bakteriozne pegavosti. Prilikom testiranja antimikrobne aktivnosti uzoraka kultivacionih tečnosti, koji sadrže i biomasu testiranih antagonista, najbolji rezultati u suzbijanju testiranih fitopatogena su postignuti primenom izolata Bacillus sp3. Sa druge strane, prilikom testiranja antimikrobne aktivnosti supernatanata oslobođenih biomase antagonista, koji sadrže samo produkovana antimikrobna jedinjenja, izolati Bacillus sp1 i Bacillus sp2 su pokazali najveću antimikrobnu aktivnost protiv fitopatogena Xanthomonas euvesicatoria. Rezultati ovog istraživanja predstavljaju osnovu za dalji razvoj bioprocesnih rešenja za proizvodnju biopesticida na bazi biomase ili antimikrobnih jedinjenja koja proizvode antagonisti roda Bacillus, a koji pokazuju visoku efikasnost u suzbijanju bakteriozne pegavosti paprike.

***Ključne reči**: antimikrobna aktivnost, paprika, bakteriozna pegavost, biomasa, antimikrobna jedinjenja, osetljivost.*

INTRODUCTION

Plant diseases caused by fungal and bacterial pathogens are still the most important source of yield losses worldwide (*Savary et al., 2012*). Bacterial plant diseases represent a more serious problem in terms of disease prevention, suppression and management since there is only a limited range of products allowed and efficient enough to suppress bacterial pathogens (*Sundin et al., 2016*). Some of the products that are usually used for the management of bacterial plant diseases include copper-based chemical pesticides and antibiotics, whose application in agriculture isn't allowed in the majority of countries (*Sundin and Wang, 2018*). The main problem when it comes to controlling bacterial pathogens is their ability to develop or acquire resistance to different chemical compounds used for their suppression in a short time period (*Sundin et al., 2016*). Therefore, the suggested alternative for the management of bacterial pathogens is the application of microbial biopesticides, which usually evince more than one mechanism of action (*Köhl et al., 2019*), making it harder for bacterial phytopathogens to develop resistance to these biocontrol agents.

Plant pathogens from the genus *Xanthomonas* represent one of the major pathogen groups responsible for massive yield losses in the wide range of host plants (*Mansfield et al., 2012*). Strains of the species *Xanthomonas euvesicatoria* are the main causative agents of pepper and tomato bacterial spot (*Potnis et al., 2015*). The symptoms of this bacterial disease in pepper plants could be observed as brownish and necrotic irregular-shaped spots in leaves, and as scab-like whitish lesions in fruits (*EPPO, 2013*), contributing to their lower market value. Furthermore, another problem is the long persistence of *Xanthomonas euvesicatoria* pathogens in the infected fields, even for 10 years (*Bashan et al., 1982*). Usual practices, when it comes to the management of pepper bacterial spot, rely on high-

quality and pathogen-free seeds and seedlings (*Šević et al., 2019*), as well as on the application of copper bactericides in combination with plant resistance activators (*Buonaurio et al., 2002*). In recent years, the application of bacterial biocontrol agents (*Sević et al., 2016*), as well as bacteriophages (*Gašić et al., 2018*), has gained significant attention in the field of pepper bacterial spot management.

The development of biocontrol products is based on an efficient and cost-effective production strategy, which implies biotechnological multiplication or production of a certain biocontrol agent, usually a highly-efficient microbial strain or some of its metabolites. The first step in this process is a search for a suitable biocatalyst, which expresses a high potential for suppression of target plant pathogens and simultaneously doesn't represent a threat to environmental biodiversity (*Mota et al., 2017*). Determination of a suitable biocontrol agent or antagonist for suppression of certain plant diseases caused by microbial phytopathogens usually relies on *in vitro* testing of its antimicrobial activity against target pathogens. Literature data could be a significant source of information when it comes to the selection of an appropriate antagonist for plant protection. Therefore, in this study several *Bacillus* strains were tested as potential antagonists for suppression of *Xanthomonas euvesicatoria* pathogens, causing pepper bacterial spots. *Bacillus* strains were selected due to several beneficial traits for application in biological control: high resistance to unfavorable environmental conditions (*Fira et al., 2018*), stability during the formulation or downstream steps in the production process (*Stamenković Stojanović et al., 2019*), as well as a genetic basis for the production of wide spectra of metabolites evincing antimicrobial activity against a wide range of plant pathogens (*Stein, 2005; Shafi et al., 2017; Fira et al., 2018*). Hence, the aim of this study was to select appropriate antagonist(s) in terms of their potential to suppress *Xanthomonas euvesicatoria* phytopathogenic strains using cultivation broth of antagonists and biomass-free supernatants as potential biocontrol agents.

MATERIAL AND METHOD

Microorganisms

Antagonistic microorganisms tested in this study were five *Bacillus* strains: two referent strains – *Bacillus subtilis* ATCC 6633 (I1) and *Bacillus cereus* ATCC 10876 (I2), and three isolates from fresh cheese – *Bacillus* sp1 (I3), *Bacillus* sp2 (I4) and *Bacillus* sp3 (I5). Isolation of these three strains was performed in the following way: successive dilutions of the fresh cheese sample (10^{-1}, 10^{-2} and 10^{-3}) were prepared and 1 mL of the last dilution was spread on the nutrient agar plate. After incubation at 28 °C for three days, colonies were picked according to the morphological traits of *Bacillus* species and subcultured to fresh nutrient agar plates. The procedure was repeated until obtaining a visually pure culture of three isolated strains. Afterwards, these strains were identified as members of the genus *Bacillus* according to their morphological and biochemical traits (*De Vos et al., 2009*). All antagonists were kept on a nutrient agar slant at 4 °C. Phytopathogenic *Xanthomonas* strains (P1, P2 and P3) were isolated using standard phytopathological techniques from pepper leaves with symptoms of bacterial spot, collected during 2015 in the cadaster municipality Pivnice, Serbia. PCR identification of phytopathogenic strains was carried out using the method described by Morreti et al. (2009). All phytopathogenic strains were identified as members of *Xanthomonas euvesicatoria* species. Phytopathogens were kept on YMA (yeast maltose agar) medium (*Pajčin et al., 2018)* at 4 °C. All microorganisms were subcultured on the media used for their preservation and incubated at 26 °C (phytopathogens) and 28 °C (antagonists) prior to further utilization.

Inoculum preparation and cultivation of antagonistic *Bacillus* spp.

After incubation of antagonistic strains at 28 °C on nutrient agar, *Bacillus* spp. were transferred to liquid media (nutrient broth) using an inoculation loop. *Bacillus* spp. inocula were incubated at 28 °C on a laboratory shaker (150 rpm, spontaneous aeration) during 48 h. After that, the inoculation of cultivation media (nutrient broth) was performed using 10% (v/v) of inocula compared to cultivation media volume (100 mL). Cultivation of *Bacillus* spp. was carried out under the same conditions as inoculum preparation, except cultivation time was 96 h.

Antimicrobial activity testing

After incubation of phytopathogenic *Xanthomonas euvesicatoria* strains at 26 °C on YMA slant, three suspensions of phytopathogenic *Xanthomonas euvesicatoria* strains were prepared using sterile saline to achieve 10^8 CFU/mL. Media containing phytopathogens were prepared by transferring 1 mL of suspension into melted and tempered (50±1 °C) YMA medium (15 mL). After pouring the medium into the Petri dish and its solidification, three paper discs (HiMedia, India) were placed on the medium surface to carry out testing of antimicrobial activity in triplicate tests for each sample (3x10 µL) against each of the phytopathogenic isolates. Samples used for antimicrobial activity assay were cultivation broths of five *Bacillus* strains (obtained after 96 hours of cultivation) and their supernatants obtained after centrifugation at 10000 rpm (13000 g) for 10 min (Rotina 380R, Hettich, Germany). Spectrophotometric measurement (600 nm, UV 1800, Shimadzu, Japan) of the optical density of cultivation broth samples was applied to determine the final concentration of *Bacillus* spp. in the samples used for antimicrobial activity assay, which was at the level of 10^8 CFU/mL. The incubation of media for antimicrobial activity assaying was carried out at 26 °C for 72 h, which was followed by the measurement of inhibition zone diameters. Furthermore, commercial streptomycin discs (Torlak, Serbia) containing 30 µg of streptomycin were also used as a positive control against each phytopathogenic *Xanthomonas euvesicatoria* strain, while sterile distilled water was used as a negative control.

Statistical data analysis

The obtained inhibition zone diameters were presented as average values (sum of the obtained values of inhibition zone diameters from triplicate tests divided by three due to the number of repetitions) with standard deviations, calculated using Microsoft® Excel 2010 software (Microsoft Corporation, USA). Statistical analysis of the experimental data was performed using Statistica 13.5 software (Tibco Software Inc., USA). Levene's test was applied to test the hypothesis of variance homogeneity, followed by ANOVA and post hoc testing using Duncan's multiple range test. All statistical analyses were performed at the significance level of 0.05.

RESULTS AND DISCUSSION

After the cultivation of five antagonists, samples of cultivation broth, as well as samples of biomass-free supernatants, were tested against three *Xanthomonas euvesicatoria* strains, isolated from diseased pepper leaves with symptoms of bacterial spot, using the diffusion-disc method. Since the testing of antimicrobial activity was performed in

triplicate tests, the results were statistically processed using Levene's test, ANOVA and post hoc Duncan's multiple range tests.

When it comes to the results of the inhibition zone diameters obtained as a result of antimicrobial activity testing using *Bacillus* spp. cultivation broth samples against *Xanthomonas euvesicatoria* strains, Levene's test with a *p*-value of 0.1377 has confirmed that there were no significant differences between repetitions for antimicrobial activity testing for each cultivation broth sample. ANOVA results, presented in Table 1, have also confirmed a significant effect of different antagonists on the obtained inhibition zone diameters against *Xanthomonas euvesicatoria* strains, with *p*-values less than 0.05.

Table 1. One-way ANOVA of inhibition zone diameters for cultivation broths of different antagonists used for suppression of Xanthomonas euvesicatoria

Effect	SS	MS	DF	F-value	*p*-value
Intercept	24875.57	24875.57	1	15318.96	**<0.0001**
Antagonist	5102.48	1020.50	5	628.45	**<0.0001**
Error	77.94	48.00	48		
SS – the sum of squares, MS – mean squares, DF –degree of freedom					

Mean values and standard deviations of the inhibition zone diameters obtained by testing of cultivation broth samples against *Xanthomonas euvesicatoria* strains are given in Table 2. These results were also processed using post hoc Duncan's multiple range test, to determine homogenous groups of independent variables at the same level of statistical significance. From the presented results it could be concluded that cultivation broth samples of the isolate *Bacillus* sp3 (I5) have shown the highest inhibitory activity against phytopathogenic *Xanthomonas euvesicatoria* strains. On the other hand, the lowest suppression of the phytopathogenic strains was achieved using the cultivation broth samples of the referent strain *Bacillus subtilis* ATCC 6633 (I1) and isolates *Bacillus* sp1 (I3) and *Bacillus* sp2 (I4), which are at the same level of statistical significance when it comes to antimicrobial activity against tested *Xanthomonas euvesicatoria* phytopathogens.

Table 2. Mean values and significance levels of inhibition zone diameters obtained using cultivation broth samples of different antagonists for suppression of Xanthomonas euvesicatoria

Antagonist	Inhibition zone diameter (mm)
I1	13.94 ± 0.81^{a}
I4	14.22 ± 0.83^{a}
I3	14.33 ± 0.41^{a}
I2	16.61 ± 1.54^{b}
I5	31.22 ± 1.56^{c}
S	38.44 ± 1.26^{d}

I1 - Bacillus subtilis ATCC 6633, I2 - Bacillus cereus ATCC 10876,
I3 - Bacillus sp1, I4 - Bacillus sp2, I5 - Bacillus sp3, S – streptomycin

The inhibition zone diameters obtained by testing *Bacillus* sp3 cultivation broth (A), streptomycin as a positive control (B) and sterile distilled water as a negative control (C) against *Xanthomonas euvesicatoria* are given in Figure 1. The second experimental stage has investigated the antimicrobial effect of supernatants (after the removal of *Bacillus* spp. biomass by centrifugation) against phytopathogenic *Xanthomonas euvesicatoria* strains. Once again, Levene's test has confirmed the homogeneity of variances with a *p*-value of 0.0876. ANOVA results are given in Table 3. These results have also confirmed a significant effect of the tested antagonist for suppression of *Xanthomonas euvesicatoria* phytopathogens using samples of cultivation broth supernatants (*p*-values less than 0.0001).

Table 3. One-way ANOVA of inhibition zone diameters for supernatants of different antagonists used for suppression of Xanthomonas euvesicatoria

Effect	SS	MS	DF	F-value	*p*-value
Intercept	8562.96	8562.96	1	9236.45	**<0.0001**
Antagonist	9091.04	1818.21	5	1961.21	**<0.0001**
Error	44.50	0.93	48		
SS – the sum of squares, MS – mean squares, DF –degree of freedom					

Afterwards, Duncan's multiple range test was applied in order to determine homogenous groups of the same statistical significance, which are given in Table 4, together with mean values and standard deviations of inhibition zone diameters resulting from testing of antimicrobial activity of supernatants against *Xanthomonas euvesicatoria*. As it could be seen in Table 4, supernatants of cultivation broths of referent strains *Bacillus subtilis* ATCC 6633 (I1) and *Bacillus cereus* ATCC 10876 (I2) didn't show any antimicrobial activity against tested phytopathogens. On the other hand, antimicrobial compounds produced by the isolates from fresh cheese have suppressed the growth of *Xanthomonas euvesicatoria* strains. The best results regarding antimicrobial activity were obtained using supernatants of *Bacillus* sp1 and *Bacillus* sp2 cultivation broths. At the same time, it can be concluded that these two isolates are at the same level of statistical significance. Therefore, any of these two isolates could be successfully applied as a biocontrol agent for the production of antimicrobial compounds effective against bacterial pepper spot causers. These results have confirmed the previously established thesis that wild strains usually express higher antimicrobial activity compared to referent strains, making the environment the richest source of microbial strains with a wide range of different biological activities (*Earl et al., 2008*). In this case, it has been also shown that wild *Bacillus* strains show better ability and genetic basis for the production of antimicrobial metabolites effective against *Xanthomonas* pathogens, in comparison to tested referent strains. Bacteria of the genus *Bacillus* are well known for their ability to produce vast antimicrobial compounds, including antibiotics (*Stein, 2005*), lipopeptides (*Ongena and Jacques, 2008*) and volatile organic compounds (*Gao et al., 2018*).

Table 4. Mean values and significance levels of inhibition zone diameters obtained using supernatant samples of different antagonists for suppression of Xanthomonas euvesicatoria

Antagonist	Inhibition zone diameter (mm)
I2	0.00 ± 0.00^{a}
I1	0.00 ± 0.00^{a}
I5	8.33 ± 1.32^{b}
I4	14.22 ± 0.97^{c}
I3	14.56 ± 1.13^{c}
S	38.44 ± 1.26^{d}

Furthermore, the sensitivity of the tested *Xanthomonas euvesicatoria* isolates towards the applied biocontrol agents has also been investigated, separately for the cultivation broth samples and the supernatant samples. ANOVA results are given in Table 5. According to the ANOVA results, the effect of the pathogen (or tested phytopathogenic *Xanthomonas euvesicatoria*

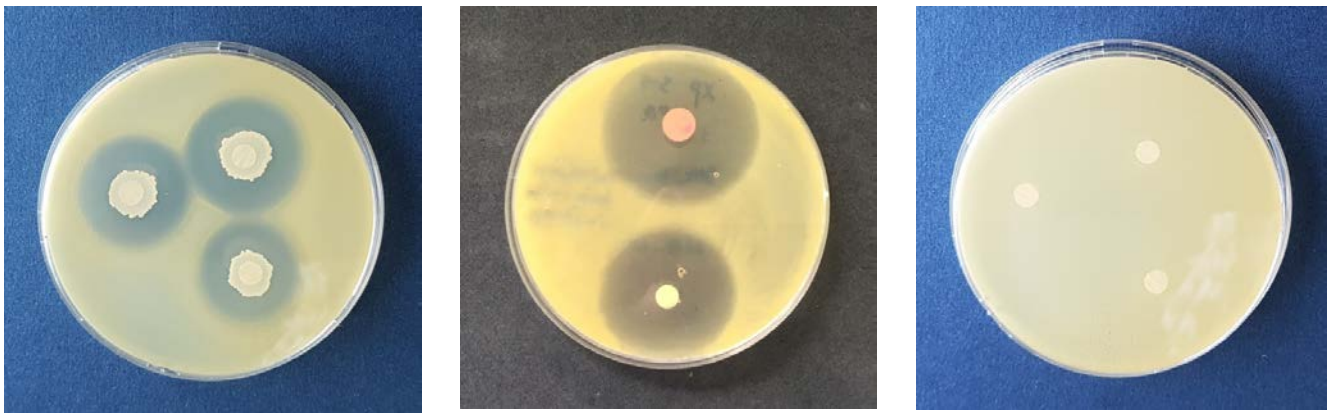

Fig. 1. Results of the antimicrobial activity assay against Xanthomonas euvesicatoria: A - Bacillus sp3 cultivation broth, B - streptomycin (positive control), C - sterile distilled water (negative control)

strain) couldn't be observed as significant when it comes to inhibition zone diameters obtained by testing both cultivation broth samples and supernatant samples as potential biocontrol agents.

ANOVA results were also confirmed by Duncan's multiple range test (Table 6), which has shown that all pathogenic isolates are at the same level of statistical significance when it comes to mean values of the obtained inhibition zone diameters. In other words, there weren't any significant differences when it comes to the sensitivity of *Xanthomonas euvesicatoria* strains to the tested *Bacillus*-based biocontrol agents. This means that the investigated biocontrol agents (cultivation broths and supernatants of the examined antagonists) could be successfully applied against all three tested phytopathogenic *Xanthomonas euvesicatoria* isolates, as the main causers of pepper bacterial spot.

Table 5. One-way ANOVA of inhibition zone diameters for different Xanthomonas euvesicatoria pathogenic strains

Effect	SS	MS	DF	F-value	*p*-value
Intercept	14688.20^{CB} 2479.02^{S}	14688.20^{CB} 2479.02^{S}	1^{CB} 1^{S}	302.63^{CB} 54.70^{S}	**<0.0001^{CB}** **<0.0001^{S}**
Pathogen	14.80^{CB} 1.64^{S}	7.40^{CB} 0.82^{S}	2^{CB} 2^{S}	0.15^{CB} 0.02^{S}	0.8591^{CB} 0.9820^{S}
Error	2038.50^{CB} 1903.33^{S}	48.54^{CB} 45.32^{S}	42^{CB} 42^{S}		

SS – the sum of squares, MS – mean squares, DF –degree of freedom
^{CB} – samples of cultivation broths, ^{S} – samples of supernatants

Table 6. Mean values of inhibition zone diameters obtained against different Xanthomonas euvesicatoria pathogenic strains

Antagonist	Inhibition zone diameter – samples of cultivation broths (mm)	Inhibition zone diameter – samples of supernatants (mm)
P2	17.33±6.28^{a}	7.20±6.66^{a}
P1	18.13±7.59^{a}	7.40±6.40^{a}
P3	18.73±6.97^{a}	7.67±7.12^{a}

CONCLUSION

The results of this study have confirmed the significant potential of different *Bacillus* strains to be used as biocontrol agents for suppression of *Xanthomonas euvesicatoria*, as principal pathogens responsible for the occurrence of pepper bacterial spot. Natural isolate *Bacillus* sp3 has shown the highest antimicrobial activity when it comes to the application of cultivation broth, while the other two natural isolates *Bacillus* sp1 and *Bacillus* sp2 have shown the highest inhibitory activity due to the production of antimicrobial metabolites, which was drawn as a conclusion from testing of antimicrobial activity of supernatants. This study has also confirmed the thesis that natural isolates usually express a higher potential for application in biological control and plant protection compared to referent isolates. Also, statistical analysis has revealed a similar sensitivity of three tested phytopathogenic isolates towards the applied biocontrol agents. Further steps will include molecular identification of the selected antagonists and their genetic basis to produce different antimicrobial metabolites characteristic for *Bacillus* species. The selection of potential antagonists done in this study represents a promising basis for further development of bioprocesses for the production of cultivation broth and antimicrobial compounds as biocontrol agents effective in suppression of pepper bacterial spot.

ACKNOWLEDGEMENT: This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia within the framework of the project 451-03-68/2020-14/200134 (2019-2020) and the Autonomous Province of Vojvodina - Provincial Secretariat for Higher Education and Scientific Research within the framework of the Short-term project of special interest for sustainable development in the AP Vojvodina entitled "Implementation of circular economy principles in technology of production of biocontrol agents" (project 142-451-3243/2020-03).

REFERENCES

Bashan, Y., Diab, S., Okon, Y. (1982). Survival of *Xanthomonas campestris* pv. *vesicatoria* in pepper seeds and roots, in symptomless and dry leaves in non-host plants and in the soil. Plant and Soil, 68, 161-170.

Buonaurio, R., Scarponi, L., Ferrara, M., Sidoti, P., Bertona, A. (2002). Induction of systemic acquired resistance in pepper plants by acibenzolar-S-methyl against bacterial spot disease. European Journal of Plant Pathology, 108, 41-49.

De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H., Whitman, W.B. (Eds.) (2009). Bergey's Manual of Systematic Bacteriology, 2nd Edition, Volume 3. Springer Science+Business Media, LLC, New York, USA.

Earl, A.M., Losick, R., Kolter, R. (2008). Ecology and genomics of *Bacillus subtilis*. Trends in Microbiology, 16, 269-275.

EPPO (2013). PM 7/110 (1) *Xanthomonas* spp. (*Xanthomonas euvesicatoria*, *Xanthomonas gardneri*, *Xanthomonas*

perforans, *Xanthomonas vesicatoria*) causing bacterial spot of tomato and sweet pepper. OEPP/EPPO Bulletin, 43, 7–20.

Fira, Đ., Dimkić, I., Berić, T., Lozo, J., Stanković, S. (2018). Biological control of plant pathogens by *Bacillus* species. Journal of Biotechnology, 285, 44-55.

Gao, H., Li, P., Xu, X., Zeng, Q., Guan, W. (2018). Research on volatile organic compounds from *Bacillus subtilis* CF-3: biocontrol effects on fruit fungal pathogens and dynamic changes during fermentation. Frontiers in Microbiology, 9, 456.

Gašić, K., Kuzmanović, N., Ivanović, M., Prokić, A., Šević, M., Obradović, A. (2018). Complete genome of the *Xanthomonas euvesicatoria* specific bacteriophage KΦ1, its survival and potential in control of pepper bacterial spot. Frontiers in Microbiology, 9, 2021.

Köhl, J., Kolnaar, R., Ravensberg, W. (2019). Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. Frontiers in Plant Science, 10, 845.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S.V., Machado, M.A., Toth, I., Salmond, G., Foster, G.D. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. Molecular Plant Pathology, 13, 614-629.

Microsoft® Excel 2010 software, v. 2010 (2010). MS Office, Microsoft Corporaton, USA (https://office.com/).

Moretti, C., Amatulli, M.T., Buonaurio, R. (2009). PCR-based assay for the detection of *Xanthomonas euvesicatoria* causing pepper and tomato bacterial spot. Applied Microbiology, 49, 466-471.

Mota, M.S., Gomes, C.B., Souza Júnior, I.T., Moura, A.B. (2017). Bacterial selection for biological control of plant disease: criterion determination and validation. Brazilian Journal of Microbiology, 48, 62-70.

Ongena, M., Jacques, P. (2008). *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. Trends in Microbiology, 16, 115-125.

Pajčin, I., Rončević, Z., Dodić, J., Dodić, S., Jokić, A., Grahovac, J. (2018). Production of biocontrol agents using *Bacillus* sp. in a laboratory scale bioreactor. Journal on Processing and Energy in Agriculture, 22, 138-142.

Potnis, N., Timilsina, S., Strayer, A., Shantharaj, D., Barak, J.D., Paret, M.L., Vallad, G.E., Jones, J.B. (2015). Bacterial spot of tomato and pepper: diverse *Xanthomonas* species with a wide variety of virulence factors posing a worldwide challenge. Molecular Plant Pathology, 16, 907-920.

Savary, A., Ficke, A., Aubertot, J.-N., Hollier, C. (2012). Crop losses due to diseases and their implications for global food production losses and food security. Food Security, 4, 519–537.

Shafi, J., Tian, H., Ji, M. (2017). *Bacillus* species as versatile weapons for plant pathogens: a review. Biotechnology & Biotechnological Equipment, 31, 446-459.

Stamenković Stojanović, S., Karabegović, I., Beškoski, V., Nikolić, N., Lazić, M. (2019). *Bacillus* based microbial formulations: Optimization of the production process. Hemijska Industrija, 73, 169-182.

STATISTICA (Data Analysis Software System), v. 13.5 (2018). Tibco Software Inc., USA (https://www.tibco.com/).

Stein, T. (2005). *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. Molecular Microbiology, 56, 845-857.

Sundin, G.W., Castiblanco, L.F., Yuan, X., Zeng, Q., Yang, C.-H. (2016). Bacterial disease management: challenges, experience, innovation and future prospects. Molecular Plant Pathology, 17, 1506-1518.

Sundin, G.W., Wang, N. (2018). Antibiotic resistance in plant-pathogenic bacteria. Annual Review of Phytopathology, 56, 161-180.

Šević, M., Gašić, K., Ignjatov, M., Mijatović, M., Prokić, A., Obradović, A. (2019). Integration of biological and conventional treatments in control of pepper bacterial spot. Crop Protection, 119, 45-51.

Šević, M., Gašić, K., Đorđevic, M., Ignjatov, M., Mijatović, M., Zečević, B., Obradović, A. (2016). Efficacy of biocontrol agents and bactericides in control of pepper bacterial spot. Acta Horticulturae, 1142, 147-150.

Received: 14. 06. 2020. Accepted: 20. 10. 2020.