In vitro Suppression Effects of Rhizobacteria against Pseudomonas syringae pv. tomato the Agent of Bacterial Speck Disease of Tomato

Gül İMRİZ*
Dicle University, Faculty of Agriculture, Department of Plant Protection, 21280 Sur Diyarbakır, Turkey
Sorumlu yazar*: gul.imriz@dicle.edu.tr
Received: 18.12.2019 Revised in Received: 23.01.2020 Accepted: 23.01.2020

Abstract
Bacterial speck caused by Pseudomonas syringae pv. tomato is significant pathogen of tomato causing economical losses in yield and quality. Bactericides for the control of such plant diseases are insufficient in some cases. Plant Promote Growth Promoting Rhizobacteria is recently considered as a potential alternative approach to the control of plant pathogens. Here, a collection of bacteria (thirty-eight in total) obtained from rhizospheric soil of different plants were examined for in vitro antagonistic effects on the Pseudomonas syringae pv. tomato growth on agar plates through dual culture assay. Seven isolates out of thirty-eight exhibited effects with varying ranges of inhibition zones from 0.1 to 0.867 cm on the growth of pathogen isolate obtained from Çumra District of Konya. Based on MALDI Biotyper classification results, all of the rhizobacterial isolates showing in vitro antagonistic actions were identified as Bacillus genera, excluding an isolate that was determined as belonged to Paenibacillus genera.

Key words: Antagonism, Bacteria, Disease, In vitro, Rhizobacteria, Tomato

Domates Bakteriyel Benek Hastalığı Etnemi Pseudomonas syringae pv. tomato’nun Üzerinde Rhizobakterilerin In vitro’ da Baskılayıcı Etkileri

Özet
Pseudomonas syringae pv. tomato’ nun neden olduğu bakteriyel benek domaste verim ve kalitede ekonomik olarak kayıplara neden olan önemli bir patojendir. Bazı durumlarda bu tür bitki hastalıklarının kontrolünde bakterisit uygulamaları yetersiz kalmaktadır. Son zamanlarda bitki patojenlerinin kontrolünde Bitki Büyümesini Destekleyen Rizobakteriler potansiyel bir alternatif yaklaşımlar olarak görülmektedir. Bu çalışmada, farklı bitkilerin rizosfer toprağından elde edilen bir bakteri koleksiyonunun (otuz sekiz adet), patojen bakterinin besi ortamında büyümesi üzerinde in vitro antagonistik etkileri iyi kültür yöntemi ile belirlenmiştir. Çalışmada kullanılan Pseudomonas syringae pv. tomato izolatı Konya-Çumra’da ağırlan üretimi yapılan domates bitkilerinden izole edilmiştir. Otuz sekiz rizobakteriyel izolattan yedi tanesi, besi üzerinde 0.1 ila 0.867 cm arasında değişen inhibisyon zonları oluşturarak Pseudomonas syringae pv. tomato’ ya karşı etkinlik göstermişlerdir. MALDI Biotyper smiflandirma sonuçlarına göre, Paenibacillus cinsine ait olduğu belirlenen bir izolat dışında in vitro’ da antagonistik etki gösteren rizobakteriizolatların tümünün Bacillus cinsine ait olduğu tespit edilmiştir.

Anahtar kelimeler: Antagonizm, Bakteri, Domates, Hastalıkt, In vitro, Rizobakteri
Introduction

Bacterial speck caused by *Pseudomonas syringae* pv. *tomato* (Pst), is a significant disease of tomato influencing economically the yield and quality worldwide including Turkey (Çınar, 1977; Wilson et al., 2002). The pathogen can maintain saprophytically in plant waste, soil and as well as on leaf surfaces. In spring, when the weather is cool and humid which favors the bacteria reproduce, the pathogen attack and causes bacterial speck disease on tomato plants. The disease symptoms can be defined with brownish-black spots surrounded by a yellow margin on leaf and ripe fruit as well as dark specks on green fruit (Preston, 2000). Particularly, in early infections, the yield could be significantly decreased due to a decline in the photosynthetic capacity of infected plant leaves. The speckled symptoms on fruit lead to reducing the market value of tomato fruits. For control bacterial speck caused by Pst, copper-based bactericides have been used, however, prolonged use of such compounds has led to copper-resistance development and hereby loses in compound effectiveness have been reported in several studies (Silva and Lopes, 1995a; Alexander et al., 1999). Also, the antibiotic resistance in the pathogen populations also has been reported by Silva and Lopes (1995b). The resistant varieties in some cases are insufficient for controlling the pathogen since the Pst has different races (Oldroyd and Staskawicz, 1998). Genetically modified tomato plants that possessive Pto/Prf genes expressions might be resistant to the races, however, it is a paradox whether these transgenic lines would be commercialized (Oldroyd and Staskawicz, 1998). The rhizosphere, the soil around the plant roots, hosts numerous of microorganisms including beneficial bacteria and has been focusing attention in recent years (Nautiyal and DasGupta, 2007). Rhizobacteria that promote plant development were first entitled as “Plant Growth-Promoting Rhizobacteria” by Kloepper and Schroth (1978). Plant growth-promoting rhizobacteria (PGPRs) can survive in the rhizosphere, the phyllosphere, or in plants. The PGPRs can boost plant growth by direct and indirect mechanisms based on the way of their impact. Indirect mechanisms involve combating the pathogen through the production of antibiotics and enzymes and the action of siderophores, inducing Systemic Resistance (ISR), and exo-polysaccharides production. Direct mechanisms are as follows; making the natural nutrition source ready to use for plants through nitrogen fixation, solubilization of phosphorus, potassium, and iron; production of siderophores; producing phytohormones namely, auxins, cytokinins and gibberellins (Ahemad and Kibret, 2014) or by producing an enzyme, 1-aminocyclopropane-1-carboxylate (ACC)-deaminase that hinders the over-secretion of ethylene in plant which is called as “plant stress ethylene” when the plant is exposed to biotic and abiotic stresses such as flooding, high temperature, organic and inorganic residual pollutants, phytopathogens, drought or salinity (Glick, 2014). Bacteria from the *Bacillus* group are microorganisms are highly adaptable to different habitats. The isolates of the genera primarily are known with the ability to produce enzymes such as proteases glucanases, cellulases, chitinases, and lipases that have a role to block the pathogen infection in the plant by demolishing the fungal and bacterial cell wall (Stein, 2005). Among the recent studies relevant with PGPRs, *Pseudomonas syringae* (synonym, *Bacillus polymyxa*) has been getting attention as a bio-control agent (Raza et al., 2008). *Pseudomonas* which separated from *Bacillus* in 1993 can enhance the plant growth by direct (e.g. production of antibiotic, hydrolytic enzymes, some other metabolites which kill directly pathogens) and indirect mechanisms (stimulating the protein secretions related to disease resistance and producing plant hormone and converting the nutrients available in soil to uptake by plants (Çakmakçı et al., 2006; Weselowski et al., 2016). The broad and prolonged application of chemical compounds in agriculture has been lead to danger for living organisms including humans and disruption of natural balance as well as ineffectiveness control based on resistant formation. Although biological control studies have been carried out for long years, more studies are still needed to find out novel eligible bio-agent in pathogen control. This study was mainly focused on determining the antagonistic properties of territorial rhizobacterial isolates on the growth of Pst in vitro which is the beginning step in the screening of a novel biocontrol agent.

Material and Method

**Isolation and identification of the pathogen**

The study was conducted in 2019. Tomato plant samples showing typical disease symptoms were collected from the open tomato production area in Çumra, Turkey. A small piece of infected tomato leaf containing infected and healthy parts (shown in Figure 1) was suspended by macerating with a small amount of sterilized distilled water (3-5 ml) in a mortar.
Loopfulls of the suspension were inoculated by streaking on King’s B agar medium (Merck, Germany) which allows separating the bacteria produce fluorescent pigment. The petri dishes were kept in an incubator adjusted to 25 °C for 48 h. The colonies grown intensively were purified on King’s B medium. Tomato seedlings (Lycopersicon esculentum cv. H-2274) were spray inoculated with suspensions prepared with purified bacterial isolates at the density of ~1x10⁸ CFU ml⁻¹. Negative control plants were treated just with sterile distilled water by spraying. For each isolate, three tomato plants were used. Inoculated plants were kept in a climate room at 25 °C and 70% humidity for 15 days to enhance the disease symptom formation. Bacteria were re-isolated from the leaf displaying bacterial speck symptom. Re-isolate bacterium was identified by a series of biochemical tests as gram reaction using the KOH method, fluorescent pigmentation, formation of levan colonies, reaction of oxidase, pectinase activity as inducing soft rot formation on potato slices, activity of arginine dihydrolase, and HR (Hypersensitivity) activity on tobacco leaves (Lelliot and Stead, 1987). The re-isolate identification was additionally confirmed by the MALDI Biotyper classification system in Plant Clinic Service at Mustafa Kemal University through service purchase.

**Rhizobacterial isolates**

Thirty-eight isolates were selected randomly from Rhizobacterial Culture Collection (Gul IMRIZ, Dicle University). The isolates were obtained from rhizosphere of different plants in Konya districts in previous study (Imriz et al., 2020). The properties belonging to the isolates including the codes, origin of plants, locations are given in Table 1.

**Table 1.** Codes, locations and origin plants of rhizobacterial isolates involved in the antagonistic activity assay.

| Isolate code | Location / district | Origin plant | Isolate code | Location / district | Origin plant |
|--------------|---------------------|--------------|--------------|---------------------|--------------|
| GP23*        | Selçuklu            | Wheat        | GP412        | Ilgın               | Wheat        |
| GP28*        | Çumra               | Nut Grass    | GP4          | Güneysınır          | Wheat        |
| GP110*       | Selçuklu            | Wheat        | GP2          | Güneysınır          | Wheat        |
| GP211*       | Selçuklu            | Wheat        | GP416        | Çumra               | Wheat        |
| GP10*        | Ilgın               | Wheat        | GP15         | Ilgın               | Wheat        |
| GP9*         | Seydişehir          | Wheat        | GP39         | Kadınhanı           | Wheat        |
| GP7*         | Selçuklu            | Barley       | GP1          | Güneysınır          | Wheat        |
| GP11         | Kadınhanı           | Barley       | GP11a        | Kadınhanı           | Barley       |
| GP196        | Cihanbeyli          | Barley       | GP29         | Ilgın               | Wheat        |
| GP122        | Kadınhanı           | Wheat        | GP16         | Güneysınır          | Wheat        |
| GP328        | Çumra               | Nut Grass    | GP25         | Selçuklu            | Wheat        |
| GP129        | Sarayönü            | Wheat        | GP13         | Güneysınır          | Wheat        |
| GP8          | Güneysınır          | Wheat        | GP20         | Selçuklu            | Wheat        |
| GP350        | Karapınar           | Wheat        | GP27         | Selçuklu            | Wheat        |
| GP3          | Yalıhöyük           | Wheat        | GP124        | Kadınhanı           | Wheat        |
| GP128        | Sarayönü            | Wheat        | GP127        | Sarayönü            | Wheat        |
| GP19         | Selçuklu            | Wheat        | GP228        | Yalıhöyük           | Wheat        |
| GP14         | Akşehir             | Wheat        | GP130        | Selçuklu            | Wheat        |
| GP6          | Seydişehir          | Wheat        | GP125        | Selçuklu            | Wheat        |
Rhizobacterial isolates and antagonistic activities

In vitro antibacterial activity of all isolates (Table 1) against Pst was screened by using the dual-culture technique (Weselowski et al., 2016) on Nutrient Agar (NA) (Merck, Germany). A hundred µl of suspension of each pathogen isolate at ~1x10⁸ CFU ml⁻¹ was spread on the NA plates by using a glass spatula. The treated plates were kept for an hour at room temperature allowed bacteria to be settled on agar medium, and those were inoculated with pure rhizobacterial isolates, which were grown for 48 hours, in three points with an equal distance to each other on agar medium by a loopful. Experiment was settled up according to randomized plot design. Three replicates were applied for each bacterial isolate. Only sterile distilled water was used instead of bacterial culture for the control plates. The experiment was conducted in sterile conditions entirely. The treated petri dishes were incubated at 25 ± 2°C for 7 days, when the control petri dishes were completely covered by pathogen. The inhibition zones were measured in cm. The isolates showed in vitro inhibition activity were identified with MALDI Biotyper classification system.

Statistical data analysis

One-way analysis of variance (ANOVA) was applied to statistically analyze the inhibition zone dimensions resulting from interactions between rhizobacterial isolates and pathogen by using software SPSS. The means were separated by the Tukey HSD test (p < 0.05).

Results and Discussion

Isolation, diagnosis and re-isolation of the pathogen

As a result of isolations on King’s B medium, fluorescent bacteria were obtained from tomato plant leaves showing typical bacterial speck symptoms. The isolates coded as ÇD displayed similar symptoms as bacterial specks on artificially inoculated tomato leaves. Isolations were made from the leaves and re-isolate was identified as Pseudomonas syringae pv. tomato by traditional bacteriological test methods as well as the MALDI Biotyper classification system. The results of traditional bacteriological tests and MALDI-Biotyper characterization for pathogen isolate are shown in Table 2.

Antagonistic activities of rhizobacterial isolates

This study exhibited that dual culture assay as a suitable method for preliminary screening of antagonistic activity and made possible the initial screen of potential bio-control rhizobacterial isolates against pathogenic bacteria Pst in tomato crop. Among thirty-eight, seven rhizobacterial isolates (namely; GP23, GP28, GP110, GP211, GP10, GP9, and GP7) displayed an ability to suppress the colonial growth of Pst on NA medium, with inhibition zones ranged from 0.1 to 0.867 cm (Table 2).

The highest inhibiting zone was formed by GP23 coded isolate with inhibition zone in size of 0.867 cm (Figure 2) whereas the minimum was obtained from GP7 (0.1 cm). The MALDI Biotyper results belonging to all isolates which showed in vitro suppression on Pst are summarised in Table 4.

According to the results, the isolates coded GP23, GP28, GP110, GP211, GP10, GP9, and GP7 were determined as Bacillus safensis, Paenibacillus polymyxa, Bacillus subtilis, Bacillus atrophaeus, Bacillus mojavensis, Bacillus pumilus, and Bacillus safensis, respectively (Table 4). The remaining thirty-one rhizobacterial isolates showed no effect against Pst (Table 3). There were statistically significant differences among the inhibition zone sizes of the isolates (F = 61.276, df = 39, P = 0.001).

Table 2. Results of Bio-chemical tests and MALDI Biotyper of pathogen isolated from tomato leaf.

| Pathogen isolate code | Bio-chemical characters | MALDI-Biotyper characterization |
|-----------------------|-------------------------|-------------------------------|
|                       | Levan type colony | Oxidase reaction | Potato rot | Arginin dehydrolase | Tobacco hyper sensitive reaction | Gram Reaction | Growth on King’s B | Score value |
| ÇD                    | +          | -          | -          | -          | +          | -          | Fluorescent     | 2.598       |
Table 3. *In vitro* antagonistic properties of the rhizobacterial isolates.

| Isolate code | Means of inhibition zones (cm) | Std. deviation | Efficiency | Isolate code | Means of inhibition zones (cm) | Std. deviation | Efficiency |
|--------------|-------------------------------|----------------|------------|--------------|-------------------------------|----------------|------------|
| GP23*        | 0,867 ±0.115                  | 86,70          | GP412      | 0,000*       |                                |                |            |
| GP28*        | 0,367 ±0.155                  | 36,70          | GP4        | 0,000*       |                                |                |            |
| GP110*       | 0,333 ±0.152                  | 33,33          | GP2        | 0,000*       |                                |                |            |
| GP10*        | 0,000*                        | 0,000          | GP15       | 0,000*       |                                |                |            |
| GP9*         | 0,100 ±0.000                  | 10,00          | GP1        | 0,000*       |                                |                |            |
| Control (Pst)| 0,000*                        | 0,000          | GP11a      | 0,000*       |                                |                |            |
| GP11         | 0,000*                        | 0,000          | GP29       | 0,000*       |                                |                |            |
| GP196        | 0,000*                        | 0,000          | GP16       | 0,000*       |                                |                |            |
| GP122        | 0,000*                        | 0,000          | GP25       | 0,000*       |                                |                |            |
| GP328        | 0,000*                        | 0,000          | GP13       | 0,000*       |                                |                |            |
| GP129        | 0,000*                        | 0,000          | GP20       | 0,000*       |                                |                |            |
| GP8          | 0,000*                        | 0,000          | GP27       | 0,000*       |                                |                |            |
| GP350        | 0,000*                        | 0,000          | GP124      | 0,000*       |                                |                |            |
| GP3          | 0,000*                        | 0,000          | GP127      | 0,000*       |                                |                |            |
| GP128        | 0,000*                        | 0,000          | GP228      | 0,000*       |                                |                |            |
| GP19         | 0,000*                        | 0,000          | GP130      | 0,000*       |                                |                |            |
| GP14         | 0,000*                        | 0,000          | GP125      | 0,000*       |                                |                |            |
| GP6          | 0,000*                        | 0,000          | GP11a      | 0,000*       |                                |                |            |

*The isolates have shown suppression on Pst growth on NA medium
Mean values followed by different superscript in a column are significantly different (p<0.05).

Figure 2. The finest inhibition zones (i.z.) with 0,867 in cm provided by isolate GP23 which identified as *Bacillus safensis* on the left and control petri completely covered by Pst on the right.

Table 4. MALDI Results for the rhizobacterial isolates which showed *in vitro* antagonistic activity.

| Isolate Code | MALDI Scores | MALDI Biotyper Results |
|--------------|--------------|------------------------|
| GP23         | 1.726        | *Bacillus safensis*    |
| GP28         | 2.101        | *Paenibacillus polymyxa*|
| GP110        | 1.762        | *Bacillus subtilis*    |
| GP211        | 2.119        | *Bacillus atrophaeus*  |
| GP10         | 1.987        | *Bacillus mojavensis*  |
| GP9          | 1.712        | *Bacillus pumilis*     |
| GP7          | 1.853        | *Bacillus safensis*    |
The results of this study are consistent with several studies that reported the bacteria in the *Bacillus* and *Paenibacillus* groups to have multifaceted properties, including antagonistic effects (Glick 2014; Weselowski et al., 2016). In this study, the presence of *Paenibacillus* and *Bacillus* was proved in the area that they had been collected from the agricultural production area in Konya-Karaman provinces (as seen in Table 1-4) where the drought is subjected. These bacteria are known as gram-positive and have the ability to produce endospores that endure even under unfavorable environmental conditions (Logan et al., 2007). The data in this study displayed isolates belong to *Paenibacillus* and *Bacillus* genera, that are gram-positive, have antagonistic potential for *Pst* and these gram-positive rhizobacterial isolates have ability to survive despite arid conditions. In the present study, the majority of successful isolates found as in vitro suppressor of *Pst* were identified as *Bacillus* (6 isolates). The bio-control potential of *Bacillus* isolates has been shown by numerous studies (Milijašević-Marčić and Todorović, 2017). Milijašević-Marčić et al., (2018) reported a study on the *in vitro* actions of *Bacillus* spp. against bacterial pathogens of tomato and pepper plants. In their study, two *B. subtilis* strains coded as B-338 and B-348 provided the highest inhibition zone on *C. michiganensis* subsp. *michiganensis* whereas the best inhibition was obtained by the treatment of *B. subtilis* strain B-319 for *X. vesicatoria*. Mirik et al., (2008) determined the *in vitro* antagonistic activities of *Bacillus* strains. The researchers observed the antagonistic suppression activity with 3 *Bacillus* strains namely; M1-3, M3-1, and against *X. axonopodis* pv. *vesicatoria*. Of the tested isolates, an isolate coded as GP28 was identified as *Paenibacillus polymyxa* (Table 4). This bacterial species has been demonstrated in previous studies for antagonism to bacterial pathogens *Xanthomonas campestris* causing agent for bacterial blight, cankers and leaf spots (Weselowski et al., 2016). On the other hand, Kim et al., (2009) indicated not all *P. polymyxa* strains have antagonistic features to pathogenic organisms, therewithal, the diversity was observed among strains in their gene clusters that encode antimicrobial peptides (Xie et al., 2016; Eastman et al., 2014). This study is the beginning of identifying potential biocontrol agents for control of *Pst* in tomato. Results obtained in the study are giving promise for biological control possibilities of the disease with potential rhizobacterial isolates as bio-control agents in tomato. Nevertheless, control of seed-borne pathogens, such as *Pst* would be initiated when the seed first being placed in soil. So that, the protection of plants against such pathogen attacks would be achieved by either seed coating or soil application with a bio-control agent which is proven for success in suppressing the pathogen. The next action has to be surely determining the efficiencies of isolates on the plants *in vivo* conditions as well as natural conditions with field experiments. Additionally, the survival of isolates in/on seed and in the soil should be revealed by detailed studies to achieve significant milestones for developing an effective bio-control agent.

References

Ahemad M., Kibret M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. Journal of King Saud University-Science, 26: 1–20.

Alexander, S. A., Kim, S. H., Waldenmaier, C. M. 1999. First report of copper-tolerant *Pseudomonas syringae* pv. *tomato* in Virginia. Plant Dis. 83:964.

Çakmakçı, R., Dönmez F., Aydın A., Şahin F. 2006. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. Soil Biology and Biochemistry. 38(6):1482-1487.

Çınar, Ö., 1977. Akdeniz Bölgesi Domateslerinde görülen bir hastalik. Bitki, 4(2), 282-288.

Eastman, A.W., Heinrichs D.E., Yuan Z.C. 2014. Comparative and genetic analysis of the four sequenced *Paenibacillus polymyxa* genomes reveals a diverse metabolism and conservation of genes relevant to plant- growth promotion and competitiveness. BMC Genom., 15:851.

Glick, B. R. 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiological Research, 169: 30–39.

İmriz G., Özdemir F., Karaca M.S., Taş M.N., Topal İ., Ercan B. 2020. Biological control potentials of rhizosphere bacteria with ACC-deaminase activity against *Fusarium culmorum* (W.G. Smith) in wheat. Zemdirbyste-Agriculture", 107:2
Kim, S.G., Khan Z., Jeon Y.H., Kim Y.H. 2009. Inhibitory effect of *Paenibacillus polymyxa* GBR-462 on *Phytophthora capsici* causing phytophthora blight in chili pepper. J Phytopathol., 157:329–37.

Kloepper, J.W., Schroth, M.N., 1978. Plant growth promoting rhizobacteria on radishes. Proc. 4th Int. Conf. Plant path. Bact., Angers, pp. 879–882.

Lelliot, R. A., Stead, D. E. 1987. Media and methods. in: Methods for the Diagnosis of Bacterial Diseases of Plants. Blackwell Scientific Publications Inc., Oxford, UK. pp. 169-199.

Logan, N.A., Popovic, T., Hoffmaster, A. 2007. *Bacillus* and other aerobic endospore-forming bacteria. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry & M. A. Pfaller (Eds), Manual of clinical microbiology, pp. 455-473, Washington, DC, USA: AMS Press.

Milijašević-Marčić, S., Todorović, B. 2017. Biological control of bacterial pathogens in horticultural systems. In Victor Green (ed.), Bio-control agents: Types, applications and research insights, pp. 1-40, New York, USA: Nova Science Publishers.

Milijašević-Marčić, S., Todorović, V., Stanojević, O., Berić, T., Stanković, S., Todorović, B., Potočnik, I., 2018. Antagonistic potential of *Bacillus* spp. isolates against bacterial pathogens of tomato and fungal pathogen of pepper. Pestic. Phytomed., 33(1): 9–18.

Mirik, M., Aysan, Y., Çınar, Ö. 2008. Biological Control of Bacterial Spot Disease of Pepper with *Bacillus* strains, Turk J. Agric. For., 32: 381-390.

Nautiyal, C., DasGupta, S.M. 2007. Screening of Plant Growth-Promoting Rhizobacteria. In: Varma A., Oelmüller R. (eds) Advanced Techniques in Soil Microbiology. Soil Biology, vol 11. Springer, Berlin, Heidelberg.

Oldroyd, G. E. D., Staskawicz, B. J. 1998. Genetically engineered broad-spectrum disease resistance in tomato. Proc. Natl. Acad. Sci., 95:10300-10305.

Preston, G. M. 2000. *Pseudomonas syringae* pv. *tomato*: The right pathogen, of the right plant, at the right time. Mol. Plant Pathol., 1:263-275.

Raza, W., Yang, R., Shen, Q.R. 2008. *Paenibacillus polymyxa*: antibiotic, hydrolytic enzymes and hazard assessment. J. Plant Pathol. 90, 419–430.

Silva, V. L., da, Lopes, C. A. 1995a. *Pseudomonas syringae* pv. *tomato* resistant to streptomycin and oxytetracycline in tomato plants treated or not with agricultural antibiotics. Fitopatol. Bras., 20:80-84.

Silva, V. L., da, Lopes, C. A. 1995b. *Pseudomonas syringae* pv. *tomato* resistant to copper in copper-sprayed tomato fields. Fitopatol. Bras., 20:85-89.

Stein, T. 2005. *Bacillus subtilis* antibiotics: Structures, syntheses and specific functions. Molecular Microbiology, 56, 845–857. doi:10.1111/j.1365-2958.2005.04587.x

Weselowski, B., Nathoo, N., Eastman, A.W., MacDonald, J., Yuan, Z.C., 2016. Isolation, identification and characterization of *Paenibacillus polymyxa* CR1 with potentials for biopesticide, biofertilization, biomass degradation and biofuel production. Microbiology, 16:24.

Wilson, M., Campbell, H. L., Ji, P., Jones, J. B., Cuppels, D. A. 2002. Biological control of bacterial speck of tomato under field conditions at several locations in North America. Phytopathology, 92:1284-1292

Xie, J., Shi, H., Du, Z., Wang, T., Liu, X., Chen, S. 2016. Comparative genomic and functional analysis reveal conservation of plant growth promoting traits in *Paenibacillus polymyxa* and its closely related species. Sci. Rep., 6:21329.