Influence of *Bacillus Spp.* Based Bioproducts on Potato Plant Growth and Control of *Rhizoctonia Solani*

Sorina DINU¹, Oana-Alina BOIU-SICUIA^{1,2*}, Florica CONSTANTINESCU¹

¹ Research-Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd., PC 013813, Bucharest, Romania.

² University of Agricultural Sciences and Veterinary Medicine – Bucharest, Faculty of Biotechnology, 59 Mărăști Blvd., PC 011464, Bucharest, Romania.

*corresponding author: sicuia_oana@yahoo.com

Bulletin UASVM Animal Science and Biotechnologies 76(1)/ 2019

Print ISSN 1843-5262; Electronic ISSN 1843-536X

DOI:10.15835/buasvmcn-asb: 2018-0022

Abstract

Some *Bacillus* based bioproducts were analyzed for their plant growth promotion and *Rhizoctonia solani* biocontrol potential in potato plants. The bioproducts were formulated as concentrated aqueous suspension, each containing one of the following plant beneficial bacteria: *Bacillus safensis* Rd.b2, *Bacillus* spp. 75.1s and Cp.b4 strains. These were applied on potato seeding material in order to evaluate plant growth promotion effects. The biocontrol efficacy was also evaluated, using *Rhizoctonia solani* DSM 63002 as plant pathogen, and Prestige 290FS as reference chemical treatment. In the plant growth-promotion experiments, several biologic parameters were biometrically evaluated. Best results regarding plant growth and vigor were obtained using CropMax, a commercial phytostimulatory product. However, the bacterial treatment with *Bacillus* spp. Cp.b4 and 75.1s showed an improved plant growth compared to the untreated control. An efficacy of 93.75% against *Rhizoctonia* dumping-off was registered when using the Prestige 290FS chemical control. Mix treatments based on this pesticide, in low dose, combined with Cp.b4 or 75.1s biocontrol strains significantly reduced the pathogenic attack, showing 85 to 87.5% efficacy. The present research demonstrated that the bacterial bioproducts based on *Bacillus* spp. 75.1s and Cp.b4 strains increase plant growth and are highly effective in controlling *Rhizoctonia* attack in potato plants.

Keywords: *Bacillus*, biocontrol, bacterial bioproducts, potato.

Introduction

Plant growth-promoting rhizobacteria (PGPR) are a group of beneficial root associated bacteria, enhancing plant growth, health and soil fertility. Some of them can present an excellent combination of useful traits for plant protection along with growth promotion (Harish *et al.*, 2009). PGPR may influence plant growth by synthesizing plant hormones or improving soil nutrients uptake through different mechanisms, such as atmospheric nitrogen fixation, phosphate and phytate solubilization, phytohormones production and siderophore synthesis, thus making nutrients more available to plants (Glick *et al.*, 2007). *Bacillus* is one of the most potential genera

among the PGPR cluster due to its spore forming ability, which increases their viability during commercial formulation and field application (Liu and Sinclair, 1993). *Bacillus* is capable of plant growth stimulation (El-Meleigi *et al.*, 2017) and some species suppress plant pathogens and pests by producing antibiotic metabolites. Several *Bacillus* spp. strains are able to stimulate plant host defenses prior to pathogen infection (van Loon, 2007), thus contributing to increased crop production. Endophytic colonization and biofilm formation of *Bacillus* and *Paenibacillus* spp. also improve the biocontrol ability (Davey and O'toole, 2000; Hallmann *et al.*, 1997; Timmusk *et al.*, 2005). Therefore, the use of such microorganisms

for plant protection and growth promotion could prevent environmental, food and feed risks resulting from chemical pesticides and fertilizers. Nowadays, some biological products based on *Bacillus subtilis* and *B. amyloliquefaciens* approved phytosanitary products for plant protection (Commission Regulation (EU) 2017/1777).

Among cultivated plants, potato (*Solanum tuberosum* L.) is mentioned as the third most important food crop after wheat and rice (International Potato Center, 2018). In 2016, Romania was ranked the 16th country worldwide, considering the harvested area, having 186233ha sown with potatoes (FAOSTAT, 2018). Taking into account the reduced potato yield obtained in our country, only 14.4 t/ha on average (FAOSTAT, 2018), we focused on some plant growth promotion products with biocontrol potential. Therefore, we analysed some *Bacillus* based bioproducts for their plant stimulatory effect and biocontrol efficacy against *Rhizoctonia solani* on potato plants.

Materials and methods

Three plant beneficial bacteria *Bacillus safensis* Rd.b2, *Bacillus* spp. 75.1s and Cp.b4 strains were formulated as concentrated aqueous suspension. These bacteria are native Romanian strains, previously isolated due to their plant beneficial potential and antifungal activity. Bacterial strains were preserved in the RDIPP microbial collection, at -80°C, in 20% glycerol. Routinely, the bacteria were grown in Luria/Miller agar (1% tryptone; 0.5% yeast extract; 1% NaCl, 1.8% agar, pH 7.2). Bacterial biomass was obtained in Luria/Miller broth, after 72h of incubation at 28°C, under rotary shaking at 150 rpm, in 100 ml flasks. The bacterial growth was aseptically harvested by centrifugation. Bacterial inoculum was formulated as concentrated aqueous suspension, diluted to 1.2×10^8 ufc/ml at application time.

Two types of experiments were performed in order to analyze the influence of beneficial bacteria inoculation on potato plants. One of these experiments was focused on plant growth promotion and the other on biological control of black scurf caused by *Rhizoctonia* disease in potato.

Potato tuber eyes were used as planting material. These are axillary buds, enclosed by a leaf scar on the potato tuber. The potato tuber

eyes were collected with a melon ball scoop (2 cm in diameter) in order to provide some adjacent nutritional tuber tissue, similar for each collected "eye". In both experiments, we used *Solanum tuberosum* L. Arizona cultivar.

In the plant growth-promotion trials, the potato tuber eyes were immersed for 15 minutes in *Bacillus* based bioproducts supplemented with 2% carboxy-methyl cellulose to improve bacterial adhesion to the plant tissue. An untreated reference was considered, where the potato tuber eyes were immersed in sterile distilled water. CropMax® ecological fertilizer was used as reference treatment for plant growth stimulation. This commercial fertilizer was used at the recommended dose of 0.1% for potato culture. The experiment was conducted in plastic pots (40/28/10 cm) filled with universal peat mixture. Plants were watered daily, maintained at 25°C during daytime and at 20°C during night. Several biometric assessments were made, five weeks after planting, regarding root and shoot length (cm), fresh and dry matter weight (g). Seedling vigor index (SVI-II) was calculated based on these results (Shruthi *et al.*, 2018), using the following formula:

SVI-II = Germination percentage x Mean seedling dry weight (g).

In the plant protection trials, *Rhizoctonia solani* DSM 63002 strain was used as fungal phytopathogen. The mycelium was grown on sterilized barley seeds, at 25°C, for two weeks. Fungal inoculum was applied to disinfected soil in 2% (w/w) concentration. Artificially infected soil was maintained at room temperature, 24h before use, to increase fungal colonization of the substrate.

Mixtures of *Bacillus* based bioproducts supplemented with 2% carboxy-methyl cellulose and combined with chemical pesticide in half dose (0.04%) were used as experimental treatments. As reference treatment, we used Prestige 290FS chemical pesticide, based on imidacloprid 140 g/l and pencycuron 150 g/l, at the recommended dose of 0.08% for potato crop.

In the plant protection experiment the planting material was placed in plastic pots filled with 125g of artificially infected soil. Two control variants were included in this experiment. One control consisted of untreated tuber eyes planted in artificially infected soil. Another untreated

Table 1. Plant growth parameters

Experimental variants	Length (cm)		Fresh weight (g)		Dry weight (g)	
	Shoot	Root	Shoot	Root	Shoot	Root
Untreated Control	30.67	8.67	3.99	0.37	0.19	0.037
CropMax 0.1%	36.00	9.00	5.56	0.65	0.26	0.060
<i>Bacillus safensis</i> Rd.b2	36.00	9.33	3.96	0.33	0.17	0.027
<i>Bacillus</i> sp. 75.1s	29.00	7.67	4.24	0.42	0.21	0.030
<i>Bacillus</i> sp. Cp.b4	36.00	9.00	5.10	0.43	0.23	0.040

Figure 1. Seedling vigor index (SVI-II)

control, with healthy potato tuber eyes, was grown in disinfected soil, without *Rhizoctonia solani* inoculum. Each variant was performed in four replicates. All variants were randomly placed in a Sanyo MLR-351H growth chamber. Plant growth conditions were programmed at 65% relative humidity and 12 hours photoperiods, at 26°C during daylight, and 20°C during night. This experiment was performed twice with similar results.

The incidence of *Rhizoctonia solani* fungus and the biocontrol efficacy were estimated according to Almoneafy *et al.* (2012), five weeks after planting. Disease incidence (DI) was calculated and recorded on a 0 to 4 scale (Park *et al.*, 2007), as follows: **0** = no symptoms; **1** = 1÷25% disease symptoms; **2** = 26÷50% disease symptoms; **3** = 51÷75% disease symptoms; **4** = 76÷100% disease symptoms or dead plants, where:

$$DI \% = \frac{\text{Disease index} \times \text{Number of diseased plants in this index}}{\text{Total number of investigated plants} \times \text{Highest disease index}} \times 100$$

The biocontrol efficacy (E%) was calculated based on the disease incidence in the control and treatment variants (Xue *et al.*, 2009), using the following formula:

$$E \% = \frac{DI_C - DI_T}{DI_C} \times 100$$

where: *EE*% = percentage of biocontrol efficacy; *DI_C* = disease incidence in control; *d* = disease incidence in the treatment variant.

Results and discussions

Five weeks old potato plants were analyzed after being treated with different biologic products. Several growth parameters were biometrically assessed, such as shoot and root length, fresh and dry weight (Tab. 1).

Best results were registered when CropMax commercial biofertilizer was used. Due to its complex formula which includes 17 amino acids, six microelements, polysaccharides, vitamins, enzymes and carotenoids. This treatment led to the highest fresh and dry weight of potato plants. Farmers that used CropMax fertilizer in Solanaceous crops mentioned an increased production of 22 to 26% (www.verdon.ro).

Among bacterial treatments, results were better when *Bacillus* sp. Cp.b4 strain was used. However, plant roots had a lower fresh and dry

Figure 2. *Rhizoctonia solani* disease symptoms in potato plants

weight, compared to plants treated with the commercial growth improver.

Compared to the untreated control, no significant differences were noticed when *Bacillus* sp. 75.1s bioproduct was applied.

The potato treatment with *Bacillus safensis* Rd.b2 strain, although induced higher shoot and longer roots, did not increase plant vigor. Due to the low fresh and dry weight of the plants it can be concluded that this treatment predisposed the plant to elongation. Therefore, such treatment should not be recommended for potato plants.

The seedling vigor index was calculated considering the plants' dry weight. Best results were obtained with the CropMax treatment (Figure 1). Bacterial treatment with Cp.b4 strain also increased the seedling vigor index compared to the untreated control. However, the other two bacterial treatments were not significantly different compared to the untreated control.

A desirable characteristic for PGPR is the capacity to produce phytohormones, like auxins. Several species of *Bacillus* have been reported to produce auxins (Calvo *et al.*, 2010; Kesaulya *et al.*, 2017). Idris *et al.*, (2007) have shown that mutants of *B. amyloliquefaciens* FZB42 with diminished levels of IAA production were less efficient in promoting plant growth. Plant growth promotion is also improved by phosphate solubilizing bacteria (Zaidi *et al.*, 2009). Several other mechanisms were developed by *Bacillus* rhizobacteria to enhance plant growth. For plant associated microorganisms an important aspect is to increase nutrients availability. Among the *Bacillus* species, *B. megaterium*, *B. subtilis*, *B. pumilus*, *B. polymyxa*, *B. sphaericus*, *B. thuringiensis* were reported to

solubilize phosphorus form insoluble inorganic phosphate or by mineralization of organic phosphorous (De Freitas *et al.*, 1997, Saeid *et al.*, 2018).

Other plant beneficial characteristics in rhizobacteria are related to their biocontrol potential. *Rhizoctonia solani* is a plant disease that attacks underground sprouts of potato plants before they emerge from the soil. At first, the fungal lesion induces superficial brown areas that have no apparent effect on plant growth. If the disease is not suppressed, the translocation of starch from leaves to the tubers is affected. The lesions expand and canker is installed. Large and sunken tissue areas become necrotic (figure 2) and the disease expands quickly. The yield is significantly reduced in such cases. *Rhizoctonia solani* could also be responsible for black scurf.

In the plant protection experiments against *Rhizoctonia* dumping-off, the chemical treatment with 0.08% Prestige 290FS revealed an efficacy of 93.75%. Reducing the chemical dose to half and supplementing this treatment with *Bacillus* spp. biocontrol bacteria we obtained an efficacy of 85% (with Cp.b4) to 87.5% (with 75.1s) in controlling *Rhizoctonia* disease (Tab 2).

Among the complex treatments, the combination of *Bacillus* sp. 75.1s suspension with the low pesticide dose was more efficient than the complex treatment containing the *Bacillus* sp. Cp.b4 strain.

Other studies performed with Prestige 290FS against *Rhizoctonia* plant pathogen mention a good efficacy both *in vitro* and *in vivo* trials. At high concentrations of this pesticide (1÷10 mg/L), the *Rhizoctonia* mycelium stops growing, and at 0.1

Table 2. *Rhizoctonia* disease control in potato

Experimental variants	E (%)	DI (%)
Negative control - Without artificial infection	-	0
Positive control - Artificial infection with <i>Rhizoctonia solani</i> DSM 63002	0	100
Chemical control - 0.08% Prestige 290FS (140 g/l imidacloprid and 150 g/l pencycuron)	93.75	6.25
Complex treatment with <i>Bacillus</i> sp. Cpb4 and 0.04% Prestige 290FS	85	15
Complex treatment with <i>Bacillus</i> sp. 75.1s and 0.04% Prestige 290FS	87.5	12.5

mg/L the mycelium reached only 2.3% the size of the control (Šafránková, 2004). However, another study mention that Prestige 290FS presented the weakest protective effect against *Rhizoctonia solani* on potato plants (Cwalinska-Ambroziak and Trojak, 2012), when analyzed compared to other pesticides (Dithane M 45 75 WG, Vitavax 2000 FS) and biocontrol agents (Polyversum). This suggests that a lower dose of Prestige 290FS should be supplemented with other chemical or biological means to control *Rhizoctonia solani* more effectively in potato plants.

Conclusions

Bacterial bioproducts based on *Bacillus* sp. 75.1s and Cp.b4 strains increase plant growth and have high efficacy in controlling *Rhizoctonia* attacks in potato plants. Between the two bacterial strains, better results were obtained for growth promotion with Cp.b4 strain compared to 75.1s strain. However, in the plant protection experiment, the complex treatment containing *Bacillus* sp. 75.1s revealed a higher efficacy against *Rhizoctonia* dumping-off. Best results were obtained in both experiments with the commercial products, CropMax as phytostimulatory product, and Prestige 290FS as plant protection product.

Acknowledgments. This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI-UEFISCDI, project number PNIII-P1-1.2-PCCDI-2017-0301, within PCCDI III.

References

1. Almoneafy AA, Xie GL, Tian WX, Xu LH, Zhang GQ, Ibrahim M (2012). Characterization and evaluation of *Bacillus* isolates for their potential plant growth and biocontrol activities against tomato bacterial wilt. *African Journal of Biotechnology*, 11(28): 7193-7201.
2. Calvo P, Ormeño-Orrillo E, Martínez-Romero E, Zúñiga D (2010). Characterization of *Bacillus* isolates of potato rhizosphere from andean soils of Peru and their potential PGPR characteristics. *Brazilian Journal of Microbiology*, 41(4): 899-906.
3. Commission Regulation (EU) 2017/1777 of 29 September 2017 amending Annexes II, III and IV to Regulation (EC) No 396/2005 of the European Parliament.
4. Cwalinska-Ambroziak B, Trojak A (2012). The effect of seed potato dressing on the severity of rhizoctoniosis and anthracnose. *Progress in Plant Protection*, 52(3): 614-618. DOI: bwmata1.element.agro-ec0902e1-0908-4ab2-b9d3-e90519fd6366
5. Davey ME, O'Toole GA (2000). Microbial Biofilms: From Ecology to Molecular Genetics. *Microbiol. Mol. Biol. Rev.*, 64(4): 847-867.
6. De Freitas JR, Banerjee MR, Germida JJ (1997). Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). *Biol Fertil Soils*, 24: 358-364.
7. El-Meleigi M.A., Omar A.F., Rogaibah A.A., Ibrahim G.H. (2017). Efficacy of Bacilli strains in growth promotion and biological control of soilborne *Rhizoctonia* and *Fusarium* on Alfalfa (*Medicago sativa* L.) and Potato (*Solanum tuberosum* L.). *Egyptian Journal of Biological Pest Control*. 27 (1): 85-92.
8. FAOSTAT (2018). <http://www.fao.org/faostat/en>
9. Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007). Promotion of Plant Growth by Bacterial Acc Deaminase. *Crit. Rev. Plant Sci.*, 26(5-6): 227-242.
10. Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997). Bacterial Endophytes in Agricultural Crops *Can J Microbiol*, 43: 895-914.
11. Harish S, Kavino M, Kumar N, Balasubramanian P, Samiyappan R (2009). Induction of Defense-Related Proteins by Mixtures of Plant Growth Promoting Endophytic Bacteria against Banana Bunchy Top Virus. *Biol Control*, 51(1): 16-25.
12. <https://www.verdon.ro/ingrasaminte-foliare-si-principicare/ingrasamant-bio-cropmax-100-natural-1-l.html>
13. Idris EE, Iglesias DJ, Talon M, Borriss R (2007). Tryptophan-dependent production of indole-3-Acetic Acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. *Mol. Plant-Microbe Interact*, 20 (6), 619-626.
14. International Potato Center (2018). <https://cipotato.org/crops/potato/>

15. Kesaulya H, Hasinu JV, Tuhumury GNC, (2017). Potency of *Bacillus* spp from potato rhizosphere as active ingredients for biostimulant formulation. *Modern Applied Science*, 11(10):74. DOI: 10.5539/mas.v11n10p74.
16. Liu ZL, Sinclair JB (1993). Colonization of Soybean Roots by *Bacillus megaterium* B 153-2-2. *Soil Biol. Biochem.*, 25(7): 849-855.
17. Park EJ, Lee SD, Chung EJ, Lee MH, Um HY, Murugaiyan S, Moon BJ, Lee SW (2007). MicroTom - a model plant system to study bacterial wilt by *Ralstonia solanacearum*. *The Plant Pathology Journal*, 23(4): 239-244.
18. Saeid A, Prochownik E, Dobrowolska-Iwanek J (2018). Phosphorus solubilization by *Bacillus* species. *Molecules*, 23:2897. DOI: 10.3390/molecules23112897.
19. Šafránková I (2004). *Rhizoctoniasolani* Kühn anastomosis Group 3 as pathogen of potato and its sensitivity to seed-fungicides. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, LII(1): 67-74.
20. Shruthi K, Balakrishna P, Sreeramu BS (2018). The effects of seed treatments on germination and other seed quality attributes of rosella (*Hibiscus sabdariffa* Var. *sabdariffa* L.). *International Journal of Science, Environment and Technology*, Vol. 7, No 1, 2018, 201 – 206
21. Timmusk S, Grantcharova N, Wagner EGH (2005). *Paenibacillus Polymyxa* Invades Plant Roots and Forms Biofilms. *Appl Environ Microbiol*, 71(11): 7292-7300.
22. Van Loon LC (2007). Plant Responses to Plant Growth-Promoting Rhizobacteria. *Eur. J. Plant Pathol.* 119(3): 243-254.
23. Xue QY, Chen Y, Li SM, Chen LF, Ding GC, Guo DW, Guo JH (2009). Evaluation of the strains of *Acinetobacter* and *Enterobacter* as potential biocontrol agents against *Ralstonia* wilt of tomato. *Biological Control*, 48(3): 252-258.
24. Zaidi A, Khan MS, Ahemad M, Oves M (2009). Plant growth promotion by phosphate solubilizing bacteria. *Acta microbiologica et immunologica Hungarica*. 56:263-84. 10.1556/AMicr.56.2009.3.6.