6e COMAPPI
COnférence sur les Moyens Alternatifs de Protection
pour une Production Intégrée
21, 22 et 23 mars 2017 – Lille Grand Palais

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EFFECT OF *BACILLUS LICHENIFORMIS* ON SEED GERMINATION OF DIFFERENT WEED SPECIES

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ABSTRACT
The effects of *Bacillus licheniformis* on seed germination and seedlings growth of several weed species (*Abutnilon theophrasti Medik., Ambrosia artemisiifolia* L., *Cuscuta campestris* Yunck., *Datura stramonium* L. and *Onopordon acanthium* L.), have been tested. Seeds of each species were germinated in water solutions containing *B. licheniformis*, in Petri dishes, while seedlings growth tested after transfer of seeds germinated in water immediately after radicle emergence. Control (seed germination/seedlings growth in water) was also included for each weed species. Germination tests were conducted in an incubator set to 25°C, in the dark. Seeds were considered to be germinated with the emergence of the radicle. Germinated seeds were counted and percentage of germination was calculated after 7 days. Also, seedlings lengths were measured 7 days after seedling transfer to bacterial solution. The obtained results shown that *B. licheniformis* inhibited *A. theophrasti*, *A. artemisiifolia*, *C. campestris* and *D. stramonium* seed germination, while effect on *O. acanthium* was opposite. Contrary to effect on germination, bacterial solution had promotional effect on seedlings growth.

Keywords: *Bacillus licheniformis*; seed germination, seedling growth; weed species.

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INTRODUCTION

Bacillus licheniformis is a Gram-positive bacterium widely distributed in the environment, which can be isolated from soils and plant material. It belonges to the *subtilis* group of genus *Bacillus* and recent taxonomic studies indicate that this bacterium is closely related to *B. subtilis* and *B. amyloliquefaciens* (Xu and Côté, 2003). *B. licheniformis* and its products can be used in many commercial and agricultural purposes. The species has been used for decades in the manufacture of industrial enzymes, like proteases used in the detergent industry as well as for dehairing and bating of leather (Eveleigh, 1981; Erickson, 1976). Also, it has exhibited potential as agent for plant diseaes biological control (Kim *et al.*, 2007; Gomaa, 2012). Many studies were shown that *B. licheniformis*, had a strong growth-promoting activity (Probanza *et al.*, 1996; Gutiérrez-Mañero *et al.*, 2001). Therefore, this bacterial species belonges to the group of PGPR (plant growth-promoting rhizobacteria), which have ability to promote growth of plants (Karlidage *et al.*, 2007) or facilitate rooting (Mayak *et al.*, 1999). Studies on PGPR have mainly focused on seed germination and seedling growth of crops with the aim to improve production in agriculture or horticulture (Bhat and Alagawadi, 1998; Carrillo-Castaneda *et al.*, 2002; Egambediyeva, 2007). However, studies of PGPR effects on weed seed germination and its young seedlings growth have been scarce (Ryu *et al.*, 2003; Vrbnicanin *et al.*, 2008; Sarić and Božić, 2009; Božić *et al.* 2014).

Weed control in modern agriculture is oriented on using many non-chemical alternative tools due to frequent development of herbicide resistant populations, as well as, increase environmental concerns and pressure to reduce pesticide use. Some of that alternative tools mean to use allelochemicals (eg. essential oils), biocontrol agent like mites, insects or parasitic plants, vegetation management, flaming etc. In that context, seed germination inhibition or stimulation can be good choice for effective reduction of weed communities in the agricultural fields. Stimulative or inhibitory effect of PGPR on seed germination or plant growth can be a component of weed control programs. Namely, thanks to its stimulative effect seedling of weed species emerge faster and more uniform and can be killed in the next step of weed control. As result of that, seedbank in the soil will be reduced and level of wideness will be decrease. Due to that reasons, the aim of this study was to determine effects of *B. licheniformis* on seed germination and seedling growth of several weed species (*Abuthilon theophrasti* Medik., *Ambrosia artemisiifolia* L., *Cuscuta compestris* Yunck., *Datura stramonium* L. and *Onopordon acanthium* L.) present in Serbian agricultural and ruderal areas.

MATERIAL AND METHODS

Seed material and bacterial strains

Seeds of five weed species (*A. theophrasti*, *A. artemisiifolia*, *C. compestris*, *D. stramonium*, *O. acanthium*) were collected during late summer and autumn 2014 near Belgrade (Serbia) in the agricultural and ruderal areas. Collected seeds stored at the room temperature (22-25°C) until use. Immediatelly before setup experiments, seeds were sterilized with sodium hypochlorite solution (1%, v/v) for 10 min and rinsed with destilled water.

The bacterium used throughout the present study was *Bacillus licheniformis*, which isolated from manure. Identification were done using API 50CH (Biomerieux, France) and API WEB (Biomerieux, France). Concentration of bacterial solution was 10^8 cells ml^{-1}.

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Seed germination assay

In the first part of experiment, seeds of selected species were germinated in Petri dishes containing 5 ml of water solution of *B. licheniformis*. Distilled water was used as control . Petri dishes incubated on 25°C in the dark during one week and number of germinated seeds were calculated. Six Petri dishes per each species were included and experiment repeated twice.

Seedling growth assay

In the second part of experiment, effect of *B. licheniformis* on seedling growth were studied for all weed species included into investigation. Seeds of each species placed in Petri dishes (30 seeds x 30 Petri dishes) with 5 ml of destilled water and incubated on 25°C until radicle emergence. High number of seeds used in order to provide enough germinated seeds due to pure germination of some species. Germinated seeds transferred to new Petri dishes containing 5ml of *B. licheniformis* solution at the moment of radicle emergence and incubated on 25°C in the dark. After 7 days, length of seedlings were measured and % of reduction were calculated.

Statistics

Differences between species were determined using t-test in statistical software STATISTICA 5.0.

RESULTS AND DISCUSSION

Seed germination assay

The germination results obtained with seeds of *A. theophrasti*, *A. artemisiifolia*, *C. campestris*, *D. stramonium*, *O. acanthium* incubated with *B. licheniformis* solution differed between species (Table 1). Bacterial solution reduced germination of almost all species (*A. theophrasti*- 44.15%, *A. artemisiifolia*- 11.03%, *C. campestris*- 100%, *D. stramonium*- 50.00). Namely, for that species percentage of germination was higher in control than in bacterial solution, but that effect was statistically very significant ($P<0.01$) only for *A. theophrasti*, *C. campestris* and *D. stramonium*. Although *A. artemisiifolia* germination was better in control, that difference was not significant ($P>0.05$). Obtained results contrary to reports of many researchers (Ryu *et al.*, 2003 ; Ahmad *et al.*, 2006; Vrbnicanin *et al.*, 2008) showing stimulative effects of *Bacillus* on seed germination and plant growth. Explanation for *Bacillus* stimulative effects is production of plant growth–promoting substances like gibberelins, indoleacetic acid, ammonia, hydrogen cyanide etc (Ryu *et al.*, 2003, Ahmad *et al.*, 2006). On the other hand, our findings are consistent with those reported by Sarić and Božić (2009), who found *Bacillus* species to have inhibitory effects on the germination of *C. campestris*. In their study, two *Bacillus* species (*B. licheniformis* and *B. pumilus*) completely inhibited *C. campestris* germination like in our investigation. Also, two (*B. amyloliquefaciens* and *B. megatherium*) other species had inhibitory effect, but that effect was little less.

Contrary to above presented results, germination of *O. acanthium* was stimulated by bacterial solution and percentage of germinated seeds was almost double in comparison with control. These results are in accordance with results of many studies which confirmed stimulative effect of PGPR on seed germination. Namely, Cassan *et al.* (2009) showed that strains of *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109 promote seed germination of corn and soybean. Similar,

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Table 1. Effect of *B. licheniformis* on germination of *A. theophrasti*, *A. artemisiifolia*, *C. campestris*, *D. stramonium* and *O. acanthium*

Species	Treatment		P
	Control	Bacterial solution	
A. theophrasti	41.67±3.12	23.33±3.00	0.003023**
A. artemisiifolia	5.62±0.61	5.00±0.40	0.698579NS
C. campestris	9.26±0.61	0.00±0.00	0.000009**
D. stramonium	10.00±1.67	5.00±2.34	00.007408**
O. acanthium	6.67±1.00	13.33±2.23	0.046322*

**) P<0.01, *) P<0.05, NS) P>0.05

Seedling growth assay

Effects of PGPR on plants (mainly crops) were studied more intensive than effects on seed germination. Many of that studies confirmed positive effect of PGPR on plant growth by production of plant hormones. Namely, a positive effect of indoleacetic acid (IAA) producing by *Bacillus subtilis* on shoot and root growth of *Dioscorea rotundata* L. were reported (Swain *et al.*, 2007), while IAA-mediated ethylene production by PGPR inoculated tomato plants increased root biomass, root hair number and root surface area (Ribaudo *et al.* 2006). Similarly, shoot growths in maize and rice were promoted by gibberellins-like substances excreted by *Azospirillum* spp. (Bolero *et al.* 2007).

Contrary to results obtained for seed germination in our research, *B. licheniformis* had positive effect on seedling growth of all studied weed species (Table 2). Seedling growth differ between studied species and it's promotion by *B. licheniformis* was very significant (P<0.01) for all species, except *C. campestris*. The most positive effect were determined for *D. stramonium*, followed by *A. artemisiifolia*, then *A. theophrasti* and *O. acanthium*, while seedlings of *C. campestris* were slightly longer (about 4%) in bacterial treatment than in control. Thanks to *B. licheniformis* seedling lenght of *D. stramonium* was more then 60% longer in comparison with control, while bacterial solution increase *A. artemisiifolia* seedlings length about 26%. Similar effect were obtained for *A. theophrasti* and *O. acanthium*, which seedling were almost 20% longer in bacterial solution then in control. Promotional effect of *B. licheniformis* on weed seedlings growth is probably result of activity of phytohormones produced by this bacteria. Gutiérrez-Mañero *et al.* (1996) have been shown *B. licheniformis* produce auxin-like compounds, while Gutiérrez-Mañero et al (2001) confirmed presence of gibberellins in its cultures. Also, Garcia *et al.* (2004) found that *B. licheniformis* increased the height of plants and the leaf area in tomato and papper.

Table 2. Effect of *B. licheniformis* on seedling growth of *A. theophrasti*, *A. artemisiifolia*, *C. campestris*, *D. stramonium* and *O. acanthium*

Species	Treatment		P
	Control	Bacterial solution	
A. theophrasti	9.25±0.37	11.08±1.03	0.000082**
A. artemisiifolia	3.25±0.07	4.11±0.12	0.000008**
C. campestris	8.97±0.53	9.02±0.75	0.563149 NS
D. stramonium	7.63±0.54	12.32±0.05	0.000016**
O. acanthium	5.22±0.43	6.14±0.03	0.00031**

**) P<0.01, *) P<0.05, NS) P>0.05; seedling lenght measured in cm.

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In conclusion, obtained results show that *B. licheniformis* can had both promoting and inhibiting effect on seed germination, while effect on seedling growth was stimulative. Therefore, this PGPR bacterium have a great potential as inhibitor or promoter of germination of weed species and as promoter of seedlings growth. But, additional studies in the field is necessary, in order to estimate practical application of this tool for weed control in the future weed management systems.

ACKNOWLEDGEMENTS

We are grateful to the Ministry of Education, Science and Technology of Republic of Serbia for supporting this investigation (Project III46008).

REFERENCES

Ahmad, F., Ahmad, I., Khan, M.S. (2008): Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiological Research, 163, 173-181.

Bhat, J.M., Alagawadi, A.R. (1998): Seed borne nature of *Azotobacter chroococcum* in chilli (*Capsicum annum*) and its role in seed germination and plant growth. Hindustan Antibiotic bulletin, 40, 20-30.

Bolero, L., Perrig, D., Masciarelli, O., Penna, C., Cassan, F., Luna, V. (2007): Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. Applied Microbiology and Biotechnology, *74*, 874–880.

Božić, D., Jovanović, Lj., Raičević, V., Pavlović, D., Sarić-Krsmanović, M., Vrbničanin, S. (2014): The effect of plant growth promoting rhizobacteria on *Datura stramonium* L., *Abutilon theophrasti* Med., *Onopordon acanthium* L. and *Verbascum thapsus* L. seed germination. Pesticides and Phytomedicine, 29, 205-212.

Carrillo-Castaneda, G., Juarez Munos, J., Peralta-Videa, J.R., Gomez, E., Tiemannb, K.J., Duarte-Gardea, M., Gardea-Torresdey, J.L. (2002): Alfalfa growth promotion by bacteria grown under iron limiting conditions. Advances in Environmental Research, 6, 391-399.

Cassan, F., Perrig, D., Sgroy, V., Masciarelli, O., Penna, C., Luna, V. (2009): *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). European Journal of Soil Biology, 45, 28-35.

Egambediyeva, D. (2007): The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Applied Soil Ecology, 36, 184-189.

Erickson, R.J. (1976): Industrial applications of the bacilli: a review and prospectus. In Microbiology. Edited by: Schlesinger D. Washington: American Society for Microbiology, 406-419.

Eveleigh, D.E. (1981): The microbial production of industrial chemicals. Scietific American, 245: 155-178.

Garcia, J.A.L., Probanza, A., Ramos, B., Palomino, M.R., Gutierrez Manero, F.J. (2004): Effect of inoculation of *Bacillus licheniformis* on tomato and pepper.Agronomie, 24, 169-176.

Gomaa, E.Z. (2012): Chitinase Production by *Bacillus thuringiensis* and *Bacillus licheniformis*: Their Potential in Antifungal Biocontrol. The Journal of Microbiology, 50, 103-111.

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Gutiérrez-Mañero, F. J., Ramos-Solano, B., Probanza, A., Mehouachi, J., R. Tadeo, F., Talon, M. (2001) The plant-growth-promoting rhizobacteria *Bacillus pumilus* and *Bacillus lichenifórmis* produce high amounts of physiologically active gibberellins. Physiologia Plantarum, 111, 206–211.

Gutiérrez-Mañero, F.J., Acero, N., Lucas, J.A., Probanza, A. (1996) The influence of native rhizobacteria on European alder (*Alnus glutinosa* [L.] Gaertn.) growth. II. Characterisation and biological assays of metabolites from growth promoting and growth inhibiting bacteria. Plant Soil, 182, 67–74

Karlidag, H., Esitken, A., Turan, M., Sahin, F. (2007) Effects of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of leaves of apple. Scientia Horticulturae, 114, 16-20.

Kim, J. H., Lee, S. H., Kim, C. S., Lim, E. K., Choi, K. H., Kong, H. G.,Kim, D.W., Lee, S.W., Moon, B. J. (2007) Biological control of strawberry gray mold caused by *Botrytis cinerea* using *Bacillus lichenifórmis* N1 formulation. Journal of microbiology and biotechnology, 17, 438-444.

Mayak, S., Tirosh, T., Glick, B.R. (1999): Effect of wild-type and mutant plant growth-promoting rhizobacteria on the rooting of mung bean cuttings. Journal of Plant Growth Regulation, 18, 49-53.

Probanza, A., Lucas, J.A., Acero, N., Gutiérrez-Mañero, F.J. (1996): The influence of native rhizobacteria on european alder (*Alnus glutinosa* [L.] Gaertn.) growth. I. Characterization of growth promoting and growth inhibiting bacterial strains. Plant Soil, 182, 59–66.

Ribaudo, C., Krumpholz, E., Cassan, F., Bottini, R., Cantore, M., Cura, A. (2006): *Azospirillum sp.* promotes root hair development in tomato plants through a mechanism that involves ethylene. Journal of Plant Growth Regulation, *24*, 175–185.

Ryu, C.M., Farag, M.A., Hu, C.H., Reddy, M.S., Wei, H.X., Pare, P.W., Kloepper, J.W. (2003) Bacterial volatiles promote growth in *Arabidopsis*. Proceedings of the National Academy of Sciences of the United States of America, 100, 4927-4932.

Serić, M., Božić, D.: Uticaj zemljišnih bakterija na klijanje semena viline kosice (*Cuscuta compestris* Yunck.) i lucerke. Zaštita bilja, 60, 227-236, 2009. (in Serbian)

Swain MR, Naskar SK, Ray RC (2007). Indole-3-acetic acid production and effect on sprouting of yam (*Dioscorea rotundata* L.) minisetts by *Bacillus subtilis* isolated from culturable cowdung microflora. Polish Journal of Microbiology, 56, 103–110.

Vrbničanin, S., Jovanovic, Lj., Bozic, D., Raicevic, V., Pavlovic, D.(2008) Germination of *Iva xanthifolia*, *Amoranthus retroflexus* and *Sorghum halepense* under media with microorganisms. Journal of Plant Diseases and Protection, XXI, 297-302.

Xu, D., Côté, J.C. (2003): Phylogenetic relationships between *Bacillus* species and related genera inferred from comparison of 3' end 16S rDNA and 5' end 16S-23S ITS nucleotide sequences. International Journal of Systematic and Evolutionary Microbiology, 53, 695-704.

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