IMPACT OF *BACILLUS MEGATERIUM* ON FERTILIZATION WITH PHOSPHOGYPSUM

ŞTEFĂNESCU IOANA ADRIANA [1*]

[1] *"Vasile Alecsandri" University of Bacau, Faculty of Engineering, Department of Chemistry and Engineering of Food Safety and Environment Protection, 157 Calea Mărăşeşti, 600115 Bacău, Romania*

Abstract: In this work rhizosphere bacteria's study present the capacity to increase plant tolerance of grown environment under conditions of added phosphogypsum (PG) (sub-product of phosphoric acid industry). The results obtains were demonstrated that the effects of *Bacillus megaterium* on the formation of the plant–rhizobium symbiosis of soybean plants depended in the majority of cases on both of the bacterial suspensions presence and PG quantitative ratios. The study shows that the bacterial strains of *Bacillus megaterium* facilitated an increase of germinated soybean plants. Also the weight and the height increase for all different soils amended with PG.

Keywords: *Bacillus megaterium*, phosphogypsum, plant growth-promoting rhizobacteria (PGPR), soybean plant

1. INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) can stimulate plant growth in association with the root systems of plants. This phenomenon appears in majority natural soils. PGPR may influence development plants in several ways. First, in soil, they can increase the formation of specific nutrients responsible for plant nutrition [1, 2]. Secondly, soil bacteria are able to stimulate growth plant by enriching the rhizosphere with a variety of growth stimulating substances such as trace elements, vitamins, enzymes, hormones, etc. [3].

Thirdly, these microorganisms release antibiotics and are therefore able to combat a series of microbial diseases [4]. All these effects resulted in the use of PGPR as a substitute for chemical fertilizers to soil, becoming more popular in ecological and sustainable agriculture.

Bacillus megaterium is one of the PGPB which is widespread in soil [5]. *Bacillus megaterium* has been industrially employed for over 50 years since it possesses some very useful and unusual enzymes, and a high capacity for the production of exoenzymes.

Genetic tools for this species include transducing phages and several hundred mutants covering the processes of biosynthesis, catabolism, division, sporulation, germination, antibiotic resistance, and recombination. Moreover, *Bacillus megaterium* owns seven plasmids which contain several unusual metabolic genes that may be useful in bioremediation [6].

* Corresponding author, email: ioana.stefanescu@ub.ro

PG pollution in soils is a world-wide problem requiring new tools for environmental management. Presently, about 120,000 tons of PG were generated as by-product from phosphoric acid in a fertilizer factory located in Bacau, Romania. PG wastes are stored in a stack occupying a surface of approximately 16 ha, less than 2 km away from the city.

In this study was tested the ability of *Bacillus megaterium*, isolated from a PG polluted soil, in order to stimulate plants occurrence, spread and growth and to increase biomasses yield.

2. EXPERIMENTAL SECTION

2.1. Isolation and identification of bacterial strains

Bacillus megaterium strain was isolated from PG polluted soil samples. The soil sample was taken from nearby PG waste dump, Bacau, Romania. The sample was heated at 80ºC for 15 min to kill the non-sporforming species. Individual bacterial strains were obtained by colony reisolation on Topping media (yeast extract – 2.5g, peptone – 2.5g, agar – 20g from 1000ml) plates at 32ºC from 24-48h and identified as *Bacillus megaterium* strains on the basis of their cell morphology and some test from confirmation – production of catalase, anaerobic growth, growth in sodium chloride, hydrolysis of starch, reduction of nitrite to nitrate, hydrolysis of casein, growth at 50-65˚C.

2.2. Phosphogypsum

PG is a residue of the phosphoric acid produced from apatites and phosphorites by extraction with sulphuric acid. PG samples (1kg) were collected in plastic bags from twelve representative points, were dried in an oven at 80ºC for 24h, homogenized, analyzed and used in growth experiments. The micronutrients content of studied samples was (mg kg^{-1}): 45 Cu; 7 Mn; 212 Zn; 1199 Fe [7].

2.3. Test soil and growth conditions

The experimental soil was collected from Vegetable Research and Development Station Bacău, from a lot intended ecological crops. The soil sample was sterilized at 121°C for 2h. Purpose of sterilization is to eliminate all microorganisms found in the soil, single monitored contribution being just for *Bacillus megaterium* strain.

Pure cultures of *Bacillus megaterium* was firstly grown in nutrient-agar [8] for 48 h at 32°C; then a loopful of culture was transferred separately to 100 mL mineral liquid medium [8], incubated at 32°C, at shaking for 24 h. After incubation, the cells were centrifuged at 5000 rpm for 10 min and washed twice with buffer phosphate pH 7.0 to remove any culture medium residue that may interfere on the growth promoting effect on soybean plants. The bacterial concentration for inoculation was at 10^8 CFU mL^{-1}.

To test the ability of *Bacillus megaterium* to solubilise PG and to increase plant growth various independent experiments were carried out in this study. There were nine treated soils with three replicates: (1) control - M (without PG and inoculums (I) application); (2) control with inoculum – M+I (without PG application); (3) - (9) – M+I+%PG application (1, 2, 3, 4, 6, 8 and 10%) equivalent to 10 - 220 t ha^{-1}.

In all the treated pots were seeded seven soybean seeds, and each seed was inoculated with 1 mL inoculum. Plants were then moved to growing chambers at 25°C, 70% RH, with 16 h of photoperiod. Plants were watered every other day with 30 ml of sterile distilled water, during the 30 days after sowing.

3. RESULTS AND DISCUSSION

The results obtains were demonstrated that the effects of *Bacillus megaterium* on the formation of the plant–rhizobium symbiosis of soybean plants depended in the majority of cases on both of the bacterial suspensions presence and PG quantitative ratios.

The influence of *Bacillus megaterium* on soybean emergence is evidenced by difference between M and M+I. Seed germination was monitored over 7 days, Figure 1 highlighting the rate of occurrence in the total number of seeds assigned to a pot.

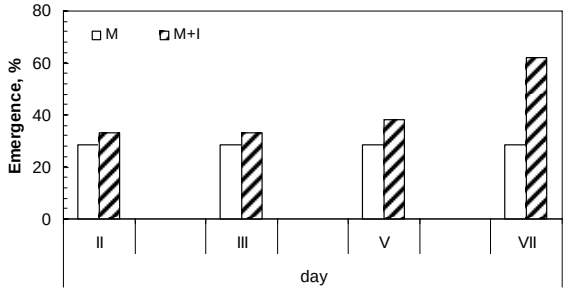

Fig. 1. *Bacillus megaterium* contribution at the rate of emergence of plant.

Lower rates of emergence of plants in the control sample (M) compared with M+I samples shows the individual contribution of *Bacillus megaterium* in germinative process. PG role in germinative process is shown in Figure 2.

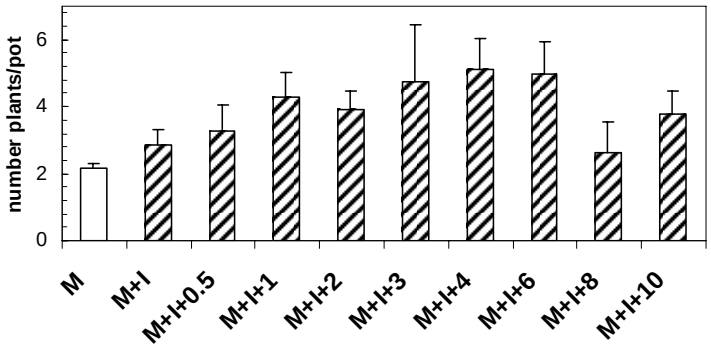

Fig. 2. Influence on PG added and *Bacillus megaterium* inoculum on emergence of soybean plants.

As a result of PG treatments was observed that the greatest number of viable plants was recorded for 0.5-6% PG additions. In the life-cycle bioassay, after 14 and 30 days, plants were measured as shoot length. The Relative Growth Rate (RGR) was calculated using measurements from days 14 and 30, based on the Radford method [9, 10]:

$$RGR=(\ln H_2 - \ln H_1)/(t_2-t_1) \quad (1)$$

where
RGR is Relative Growth Rate (cm day^{-1});
H_1 - length in t_1 (cm); H_2- length in t_2 (cm);
t_1 - number of days in the first time measurement;
t_2 - number of days in the second time measurement.

After 14 days of the PG treatments, the height plants were significantly different between the control (M) and other treatments (Figure 3).

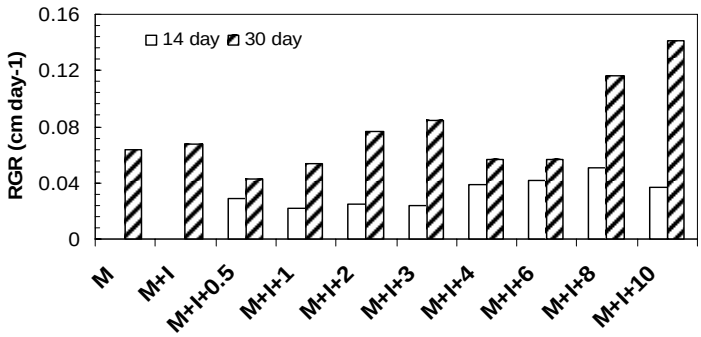

Fig. 3. Relative Growth Rate of soybean plants.

Significant correlations were observed between the PG addition and RGR, showing an increase of RGR with increasing PG concentrations in the soil. So, in the first part of the vegetative period was obtained a Pearson correlation $r = 0.83$ at $p < 0.01$ and for the end of this period $r = 0.761$ at $p < 0.05$.

The bacterial inoculation also increased plants weight compared to the control plants, but the bacterial effect was more evident in PG treatments where increased plant water content (Table 1). This bacterial effect is relevant for plants grown because it allows nutrient translocation.

Table 1. Biomass of soybean plants augmented in PG treatments with *Bacillus megaterium* contribution.

Samples	PG, [%]	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})
M	-	0.713	0.044
M+I	-	0.788	0.062
M+I+0.5	0.5	0.921	0.060
M+I+1	1	1.168	0.066
M+I+2	2	1.167	0.065
M+I+3	3	1.215	0.063
M+I+4	4	1.215	0.064
M+I+6	6	1.224	0.062
M+I+8	8	0.977	0.068
M+I+10	10	0.716	0.057

4. CONCLUSIONS

This work demonstrates that *Bacillus megaterium* has played an important role in rhizosphere colonization and in translocation levels of nutrient elements from PG to plants. The bacterial strains *Bacillus megaterium* facilitated an increase of germinated soybean plants. Also the weight and the height augment for all different soils amended with PG.

The analysis of statistical results showed an increase in biomass at low PG amendment ratio (up to 6%). On the other hand, soybeans plants rise in the same rate; however the accumulated control biomass is about two times lower than the ones obtained in the case of soybean plants grown in 1-6% PG treatments. As a perspective for this study, we intend to use the plants, in the presence of *Bacillus megaterium*, for bioremediation of PG contaminated soils.

REFERENCES

[1] Nowak, J., Benefits of in vitro “biotization” of plant tissue cultures with microbial inoculants, In Vitro Cellular & Developmental Biology - Plant, vol. 34, no 2, 1998, p. 122-130.
[2] Vessey, J. K., Plant growth promoting rhizobacteria as biofertilizers, Plant and Soil, vol. 255, no. 2, 2003, p. 571-586.
[3] Qin, S., Xing, K., Jiang, J.-H., Xu, L.-H., Li, W.-J., Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria, Applied Microbiology and Biotechnology, vol. 89, no. 3, 2011, p. 457-473.
[4] Chan, Y.-K., Savard, M. E., Reid, L. M., Cyr, T., McCormick, W. A., Identification of lipopeptide antibiotics of a *Bacillus subtilis* isolate and their control of Fusarium graminearum diseases in maize and wheat, BioControl, vol. 54, no. 4, 2009, p. 567-574.
[5] Ali, B., Sabri, A. N., Ljung, K., Hasnain, S., Quantification of indole-3-acetic acid from plant associated *Bacillus* spp. and their phytostimulatory effect on Vigna radiata (L.), World Journal of Microbiology and Biotechnology, vol. 25, no. 3, 2009, p. 519-526.
[6] Vary, P. S., Biedendieck, R., Fuerch, T., Meinhardt, F., Rohde, M., *Bacillus megaterium*—from simple soil bacterium to industrial protein production host, Applied Microbiology and Biotechnology, vol. 76, no. 5, 2007, p. 957-967.

[7] Stefanescu, I. A., Gavrila, L., Mocanu, R. D., Olariu, R. I., Arsene C., Posibilităţi de bioremediere cu ajutorul *Bacillus megaterium* a unor medii bogate în fosfoghips cu conţinut de metale grele, Rev Chim (Bucuresti), vol.62, no.2, 2011, p. 245-249.
[8] Stefănescu, I. A., Mocanu, R., Surdu, Ş., Micronutrients influence on growth cell biomass a strain *Bacillus megaterium* in soil, *Agronomical Research in Moldavia*, vol. 43, nr. 3(143), 2010, p. 61-67.
[9] Hoffmann, W.A., Poorter, H., Avoiding bias in calculations of relative growth rate, Annals Botany, vol. 80, 2002, p. 37–42
[10] Radford, P.J., Growth analysis formulae- their use and abuse, Crop Science, vol. 7, 1967, p. 171–175.