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"Gheorghe Asachi" Technical University of Iasi, Romania

EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA ON *Zea mays* DEVELOPMENT AND GROWTH UNDER HEAVY METAL AND SALT STRESS CONDITION

Annamária Becze[1,2], Éva-Boglárka Vincze[2,3], Hilda-Mária Varga[2], Mara Gyöngyvér[2*]

[1]Politehnica University of Bucharest, Faculty of Applied Chemistry and Material Sciences, Department of Analytical Chemistry and Environmental Engineering, 1-7 Polizu Street, 011061- Bucharest, Romania
[2]Sapientia Hungarian University of Transylvania, Faculty of Economics, Socio-Human Sciences and Engineering, Libertății sq., 1, 530104 - Miercurea Ciuc, Romania
[3]University of Pécs, Faculty of Science, Doctoral School of Chemistry, Vasvári Pál Street 4., 7622 - Pécs, Hungary

Abstract

Plants, being sessile organisms are exposed to a variety of biotic and abiotic stresses. Bacteria, living in the rhizosphere of the plants can be beneficial, exhibiting plant growth promotion effect and inducing the stress tolerance of the plant. Therefore, studies regarding the role of plant growth promoting rhizobacteria (PGPR) in the stress management of the plants have received increasing attention. In the present study, we studied the stress tolerance and beneficial trait maintenance of 30 PGPR strains under stress condition. From a total of 17 heavy metal and salt stress tolerant bacterial strains, two Cd and Zn tolerant PGPR strains *Viridibacillus* sp. (BP13) and *Defltia acidovorans* (BP12) as well as two salt tolerant strains *Pantoea agglomerans* (8G/3) and *Serratia fonticola* (BB17) were selected for plant experiments. When heavy metal and salt stress were applied, the beneficial effect of PGP bacterial inoculation on maize plant growth and development was confirmed only sporadically. Stress mitigation was observed in the case of *Viridibacillus* sp. (BP13) and *Defltia acidovorans* (BP12) strains (0.1 mM Cd treatment), *Serratia fonticola* (BB17) (until 3 g/L NaCl) and *Pantoea agglomerans* (8G/3) (5 g/L NaCl). Despite the sporadically observed beneficial effect of the PGP bacterial inoculation on plant growth and development; a higher guaiacol peroxidase (GPOX) activity observed under *Viridibacillus* sp. (BP13) bacterial inoculation in the presence of heavy metal stress, as well as *Pantoea agglomerans* (8G/3) and *Serratia fonticola* (BB17) inoculation in the case of salt stress revealed that PGP bacterial strains increased the plant tolerance to abiotic stressors.

Keywords: abiotic stress, maize, plant growth, plant growth promoting rhizobacteria (PGPR)

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1. Introduction

Rhizobacteria inhabit the nearest region of the plant root system, the so called rhizosphere. There is a complex chemical signaling between bacteria and root hairs, leading to a mutually beneficial coexistence. While metabolites excreted by the root hairs are the main nutrient source for the bacteria, while several bacterial driven mechanisms such as nitrogen fixation, phosphate solubilization and mobilization, as well as indole-3-acetic acid, siderophore, ammonia and biofilm (exopolysaccharide) production promote plant growth (Nihorimbere et al., 2010; Odelade and Babalola, 2019).

Plants, being sessile organisms, are exposed to a range of stressors arising from the environment. According to the origin the stress factors affecting crop plants can be divided into two types, biotic and abiotic factors. Attacks by various viral, fungal or bacterial pathogens, nematodes and herbivores are

*Author to whom all correspondence should be addressed: e-mail: maragyongyver@uni.sapientia.ro; Phone: +40-266-314657; Fax: +40-266-372099

responsible for biotic stresses. The basic abiotic components in the agricultural ecosystem that have an influence on plants, are the soil water status, temperature, pH, salinity and toxic metal content (Enebe and Babalola, 2018; Khan et al., 2019b). Plants sense the environmental factors through stress specific transmembrane proteins and sphingolipids, these mechanisms being followed by serial cellular signaling pathways in order to acclimatize to adverse conditions (Lamers et al., 2020).

Heavy metals occur naturally in agricultural soils, but higher concentrations are attributable to anthropogenic activities such as: the intensive use of different agrochemicals (phosphate fertilizers and pesticides), sewage sludge and wastewater application in agricultural practice. Most of the heavy metals are vital for plant growth and metabolism, and are considered essential (Cu, Co, Cr, Fe, Ni, Zn, Mn, Mo), whereas others have no biological functions and are considered nonessential (Cd, Hg, Pb, Se, As). The top most widespread environmentally toxic metals are As, Pb, Cd, and Hg (Prabhakaran et al., 2016). In excess both essential and nonessential metals are toxic, if they are taken up through root system or foliar absorption. Heavy metal stress causes chlorosis, growth inhibition and yield depression in crop plants (Štolfa et al., 2015). Above a certain optimum zinc (Zn) is toxic for plants leading to leaf chlorosis and growth inhibition (Anwaar et al., 2015), and cadmium (Cd) toxicity causes morphological, physico-chemical and structural changes in plants, inhibiting the plant root formation, photosynthesis, nutrient uptake and inducing oxidative stress (Mitra et al., 2018).

On the other hand, salinization is a devastating environmental stress factor in agroecosystems that markedly limits plant growth and productivity. About 6% of the world total land area is affected by salt stress (Hasanuzzaman et al., 2018), causing a decrease in the productivity of important cereal crops like wheat, maize, rice, and barley up to 70% (Etesami and Beattie, 2018). The effects of salinity stress on plants include both the osmotic and ionic stress. Salt-induced osmotic stress breaks up the plant water balance and inhibits the cell expansion and division. Ionic stress disrupts nutrient homeostasis, which leads to other stress factors associated with cellular functioning. Thus, plants under salt stress are affected in germination, growth, and development, due to a reduced chlorophyll concentration, low stomatal conductance and unusual transpiration rate (Enebe and Babalola, 2018; Hasanuzzaman et al., 2018; Khan et al., 2019b; Mitra et al., 2018).

Plant growth promoting rhizobacterial strains (PGPR) can maintain their beneficial properties under stress conditions, supporting directly or indirectly the wellbeing of plants. Several bacterial strains due to their metabolic activity produce a variety of substances (indole acetic acid, siderophore, deaminase and phosphate solubilizing enzyme) that protect plants. Others, able to produce exopolysaccharide and form biofilm sustain and activate the systemic resistance of plant (Basu and Kumar, 2020; Enebe and Babalola, 2018; Goswami and Deka, 2020). Heavy metal resistant plant growth promoting rhizobacteria belonging to the following genera: *Acinetobacter* sp. (Mindlin et al., 2016), *Azotobacter* sp. (Diaconu et al., 2020), *Arthrobacter* sp., *Kocuria sp., Paenibacillus* sp., *Pantoea* sp., *Rhizobium* sp. (Manoj et al., 2020), *Braevibacillus* sp., *Kluyvera* sp. (Prasad et al., 2019), *Delftia* sp. (Braña et al., 2016), *Enterobacter* sp. (Pramanik et al., 2018), *Klebsiella* sp., *Pseudomonas* sp., *Serratia* sp., *Strenotrophomonas* sp. (Mitra et al., 2018) and *Mitsuaria* sp. (Vincze et al., 2018), induced heavy metal tolerance in different plant species. The salt tolerance of various plants was induced by salt tolerant plant growth promoting rhizobacteria belonging to genera like: *Alcaligenes* sp., *Agrobacterium* sp. *Enterobacter* sp., *Klebsiella* sp., *Microbacterium* sp. (Egamberdieva et al., 2019), *Azospirillum* sp., *Bacillus* sp., *Rhizobium* sp., *Pseudomonas* sp. (Enebe and Babalola, 2018), *Lysinibacillus* sp. (Damodaran et al., 2018), *Serratia* sp. (Khan et al., 2019a) and *Pantoea* sp. (Gond et al., 2015).

The aim of our research was to determine the effect of abiotic stress resistant PGP bacteria on the early development of maize plant under heavy metal and salt stress.

2. Material and methods

2.1. Bacterial strains and their abiotic stress tolerance

A number of 30 PGP bacterial strains were previously isolated from soil originated from the Ciuc Mountains (György et al., 2010), the Borsáros-raised bog (Szentes et al., 2013) and Cristuru Secuiesc (Tamás et al., 2012) region were tested for their tolerance toward various abiotic stressors like salinity and heavy metals. The studied bacterial strains belong to the following species: *Achromobacter* sp. (ST5, ST5/4), *Acinetobacter lwoffii* (4CZR, 2CNS), *Bacillus mycoides* (BB6), *Cedecea neteri* (P4), *Delftia acidovorans* (BP12), *Delftia lacustris* (6BS), *Lysinibacillus* sp. (BP24), *Strenotrophomonas* sp. (E3), *Strenotrophomonas rhizophila* (BB21), *Serratia* sp. (BE11), *Serratia fonticola* (BB17, P5), *Serratia phymutica* (BE5, 9BS), *Serratia liquefaciens* (ST1/1), *Pantoea agglomerans* (8G/3), *Phyllobacterium myrsinacearum* (T5 10^{-2}/1), *Pseudomonas* sp. (BP8, BP20, BP23), *Pseudomonas jersenii* (BE20), *Pseudomonas fluorescens* (BE8), *Pseudomonas koreensis* (BP15), *Pseudomonas mohnii* (ST2/1), *Variovorax paradoxus* (14 G2, T12 10^{-3}/2, T12 10^{-3}/3) and *Viridibacillus* sp. (BP13).

The abiotic stress tolerance of the bacterial strains was tested using microtiter plate screening. The utilized Nutrient broth for abiotic stress tolerance testing (meat extract 1 g/L, yeast extract 2 g/L, peptone 5 g/L, NaCl 5 g/L, pH 7.4) was supplemented with salt (1-10 g/L NaCl) or heavy metal (0.1 mM, 0.5 mM, 1 mM, 5 mM, 10 mm, 15 mM Cd^{2+} and 0.5 mM, 1 mM, 5 mM, 10 mM, 15 mM, 25 mM Zn^{2+}) up to the required concentration. A quantity of 200 µl of

Nutrient broth was inoculated with 4 µL bacterial suspension (OD_{595} = 0.3), then incubated at 28 °C, 150 rpm. Four replicates for each treatment was realized. The growth of bacterial strains was followed (absorption at 595 nm) during 70 hours in the presence of different salt or heavy metal concentrations.

2.2. Testing PGP traits under stress condition

After the selection of the salt and heavy metal tolerant bacterial strains, we examined the PGP traits of the 17 selected strains in the presence of stress conditions: inorganic phosphorus mobilization ability, siderophore (Laslo et al., 2012), exopolysaccharide (EPS) (Fujishige et al., 2006), and indole-3-acetic acid production (Szentes et al., 2014). Each medium and broth was supplemented with salt (1 g/L, 3 g/L, 5 g/L, 7 g/L, 9 g/L) or heavy metal (0.1 mM Cd^{2+}, 0.5 mM Cd^{2+}, 0.5 mM Zn^{2+}, 1 mM Zn^{2+}, 0.1 mM Cd^{2+}+0.5 mM Zn^{2+}) in different concentrations.

The inorganic phosphorus mobilization assay was performed using Pikovskaya's medium (yeast extract 0.5 g/L, glucose 10 g/L, Ca_3PO_4 5 g/L, ammonium sulphate 0.5 g/L, KCl 0.2 g/L, $MgSO_4$ 0.1 g/L, $MnSO_4$ 0.0001 g/L, $FeSO_4$ 0.0001 g/L, agar 15 g/L, pH 7). Overnight grown bacterial cultures were point inoculated (10 µL, OD=0.3) in three replicates using a micropipette. The plates were incubated at 28 °C, for 3 days. Phosphate solubilization was observed based on the detection and measurement of a clear (halo) zone around the bacterial colony.

The siderophore production of bacterial strains in presence of salt or heavy metals was performed using chrome azurol S (CAS) medium (piperazine 6 g/L, NaOH 0.6 g/L, proteose-peptone, 15 g/L, $MgSO_4 \cdot 7\ H_2O$ 15 g/L, K_2HPO_4 1.5 g/L, glycerol 10 mL/L, agar 20 g/L, chrome-azurol S (CAS) dye 60.5 mg/L, $FeCl_3 \cdot 6\ H_2O$ 10 mg/L, hexadecyl-trimethyl-ammonium bromide (HDTMA) 72.9 mg/L, pH 6.5). Overnight grown bacterial cultures were point inoculated (10 µL, OD=0.3) in three replicates using a micropipette. The plates were incubated at 28 °C, for 3 days. The diameter of the orange halo formed around the bacterial colonies, indicating the siderophore production, was measured.

The exopolysaccharide production was determined using microtiter plate assay. The bacterial strains were cultured in 200 µL Nutrient broth (meat extract 1 g/L, yeast extract 2 g/L, peptone 5 g/L, NaCl 5 g/L, pH 7.4), for 24 hours at 28°C, followed by the absorbance reading at 595 nm in order to determine the growth rate of bacterial strains. The medium was removed, and the biofilms were stained with 0.01% crystal violet for 20 minutes. After three washing steps with distilled water, the dye was solubilized with 96% ethanol (10 minutes), and quantified measuring the absorbance at 570 nm.

The detection of phytohormone (indole-3-acetic acid) production was realized by spectrophotometric measurement. Bacterial strains were grown in 5 mL TSB (Tryptone-Soy Broth) broth (15 g/L tryptone, 5 g/L soy peptone, 5 g/L NaCl, tryptophan 10 mg/mL), at 28 °C and 150 rpm for 72 hours. 1 mL of culture supernatant was mixed with 2 mL of Salkowsky reagent (300 mL of 98% H_2SO_4, 15 mL of 0.5 M $FeCl_3$, 500 mL of distilled water), then incubated for 30 minutes in the dark at room temperature. The absorbance of the samples was measured at 570 nm, and the auxin concentration calculated using a standard curve.

2.3. Plant experiments

The effect of PGP inoculation was tested on crop plants under stress conditions. The plant seeds (*Zea mays*) were surface sterilized, germinated in Petri dishes on wet filter paper in dark at 28°C for 5 days. After germination there were sown in sterilized soil (121°C, 20 minutes, 2 times) into sterile polypropylene boxes (n=20 each treatment) and then inoculated with bacterial suspensions (0,5 mL/seed, OD_{590}=1.5). The soil was treated with different concentrations of heavy metals (0.1 mM and 0.5 mM Cd^{2+}, 0.5 mM and 1 mM Zn^{2+}, and their combinations) and salt (1-9 g/L NaCl). Seedlings were placed for 15 days in a plant growth chamber (Sanyo MLR-351 Versatile Environmental Test Chamber) under controlled conditions: 22°C, 70% relative humidity, 12 h/day 2500 lx light. After harvesting, the length and weight of plant shoot and root were measured.

2.4. Chlorophyll content measurements

The total chlorophyll (chlorophyll-a, chlorophyll-b) content was determined spectrophotometrically. An amount of 0.15 g fresh leaf tissue was weighed and homogenized by a mortar in ammonia-acetone solution (1 L of 80% acetone, 1.5 mL of 25% ammonia solution). The plant extract was centrifuged at 9850 g for 5 minutes in centrifuge tubes, and the supernatant was filled into volumetric tubes and made up to 5 mL with ammonia-acetone. The absorbance of the samples (n=3, for each treatment) was measured with a spectrophotometer (HACH DR 6000) in a quartz cuvette at the following wavelengths: 800 nm, 730 nm, 664 nm and 647 nm respectively. The amount of chlorophyll-a and chlorophyll-b was determined from the absorbance values according to the formula proposed by Porra et al. (1989).

2.5. Protein extraction and antioxidant enzyme activity measurements

The protein extraction was performed from 0.1 g of frozen plant biomass (shoot). The samples were transferred into 2 mL tubes supplemented with 1.4 mm ceramic beads in 1 mL of QB buffer (for 100 mL: 5 mL 2 M KPO_4 (pH 7,8), 200 µL 0.5 M EDTA, 1 mL Triton X-100, 12.5 mL 80% glycerol, 81.1 mL distilled H_2O, 100 µL 1 M DTT before use). Cells were disrupted mechanically using MP FastPrep-24 mill (five times for 30 s at 5 m/s speed). The homogenized plant extract was centrifuged at 27500 g

for 30 minutes at 4°C, and the supernatant was pipetted into a new 1.5 mL centrifuge tube and stored at -20 °C until use. Prior to use, the plant extracts were centrifuged again (27500 g, 30 min, 4°C). The total protein was determined using Bradford method (Bradford, 1976).

The activity of guaiacol peroxidase enzyme (GPOX) was determined spectrophotometrically at room temperature at a wavelength of 436 nm until the end of reaction. The reaction mixture contained 50 µL crude protein extract, 1225 µL solution containing 0.2 mM phosphate buffer (pH 7.5), 20 mM H_2O_2 and 50 mM guaiacol (Cavalcanti et al., 2004). One enzymatic unit was defined as the amount of enzyme producing 1 mmol of tetra guaiacol per minute. Specific activity was expressed in U/mg protein.

2.6. Statistical analysis

Data presentation and statistical analysis included the use of Microsoft Office Professional Plus 2013 and Past.exe 3.24 statistical program. To compare datasets between groups (treatments and control) One-way ANOVA was used, followed by Tukey test.

3. Results and discussion

3.1. Abiotic stress tolerant bacterial strains

A number of 30 PGP bacterial strains isolated from soil originated from the Ciuc Mountains, the Borsáros-raised bog and the Cristuru Secuiesc region were tested for their salinity and heavy metal tolerance. The following ten heavy metal tolerant strains: *Serratia liquefaciens* (ST1/1), *Serratia* sp. (BE11), *Serratia phymuthica* (BE5), *Pantoea agglomerans* (8G/3), *Acinetobacter lwoffii* (4CZR), *Serratia fonticola* (BB17), *Acinetobacter lwoffii* (2CNS), *Viridibacillus* sp. (BP13), *Delftia acidovorans* (BP12), *Pseudomonas koreensis* (BP15) from the 30 tested were selected for further analysis. However, bacterial strains from most genera were described earlier as heavy metal tolerant PGPRs, the present study being the first in which this fact is proved for *Viridibacillus* sp. The next ten bacterial strains were selected as salt tolerant: *Serratia phymutica* (9BS), *Pseudomonas mohnii* (ST2/I), *Cedecea neteri* (P4), *Serratia fonticola* (P5), *Pantoea agglomerans* (8G/3), *Lysinibacillus* sp. (BP24), *Pseudomonas fluorescens* (BE8), *Serratia* sp. (BE11), *Pseudomonas* sp. (BP23) and *Serratia fonticola* (BB17). Two bacterial strains *Pantoea agglomerans* (8G/3) and *Serratia fonticola* (BB17) proved to be both heavy metal and salt tolerant. Many PGP bacterial genera were described previously, but no data was found in scientific literature about *Cedecea neteri*.

3.2. PGP traits under abiotic stress

The heavy metal tolerant strains were further tested for inorganic phosphorus solubilization, siderophore, exopolysaccharide (EPS) and indole acetic acid (IAA) production in the presence of Zn (0.5 mM, 1 mM) and Cd (0.1 mM, 0.5 mM) and their combination (0.5 mM Zn + 0.1 mM Cd). From six bacterial strains capable of inorganic phosphorus solubilisation only *Serratia* sp. (BE11) strain was able to maintain this trait under heavy metal stress, regardless of the used concentrations. Siderophore production capacity of the selected bacterial strains was less affected by the heavy metal stress; it was preserved in all eight strains. Each bacterial strain was able to produced EPS in different quantities. In the case of four bacterial strains at higher heavy metal stress the EPS production was inhibited. From eight IAA producing bacterial strains three were able to produce in higher quantities despite the applied stressors (Fig. 1).

The salt tolerant strains were further tested for their plant growth promoting traits (inorganic phosphorous solubilization, siderophore, indole acetic acid and exopolysaccharide) and also their production in the presence of different concentrations of NaCl (1 g/L, 3 g/L, 5 g/L, 7 g/L and 9 g/L). Five strains were capable of inorganic phosphorus solubilisation, but only one *Pantoea agglomerans* (8G/3) maintained this trait under salt stress conditions. The siderophore and IAA production capacity of the selected bacterial strains was less affected by the heavy metal stress (maintained in eight strains both for siderophore and IAA). The EPS production was proved in eight bacterial strains, the PGP trait was influenced by the salt stress in case of two bacterial strains (Fig. 2).

Based on our results, most of the bacterial strains maintained their plant growth promoting properties, even in the presence of high heavy metal or salt concentrations, thus having potential in sustainable agriculture. Taking into consideration the above presented data obtained for PGP traits under abiotic stress, four bacterial strains were selected for further study, two heavy metal tolerant: *Viridibacillus* sp. (BP13), *Delftia acidovorans* (BP12) and two salt tolerant *Serratia fonticola* (BB17) and *Pantoea agglomerans* (8G/3)).

3.3. The effect of bacterial inoculation on plant growth under abiotic stress

The effect of two selected PGP rhizobacterial strains: *Delftia acidovorans* (BP12) and *Viridibacillus* sp. (BP13), on maize (*Zea mays*) growth, was evaluated under heavy metal stress. Without heavy metal stress the two bacterial strains slightly contributed to plant growth, increasing the total length of the maize plants with 4% (*Delftia acidovorans* (BP12)) respectively and 15% (*Viridibacillus* sp. (BP13)), but the differences were statistically non-significant (Fig. 3). However significant differences were recorded in the case of 0.1 mM Cd treated plants, where *Delftia acidovorans* (BP12) strain increased with 26% (59.28±8.93, p=0.049), whereas *Viridibacillus* sp. (BP13) strain increased with 33% (62.57±3.79, p=0.002) the total length of maize plants.

In order to assess the role of PGP bacteria in plant growth and development under salt stress, two strains were used for maize plant inoculation: *Pantoea agglomerans* (8G/3) and *Serratia fonticola* (BB17). None of the strains increased plant growth of the maize plant without stress conditions. The increasing salt concentration caused a decrease in the total length of the plant with and without bacterial inoculation (Fig. 4).

A statistically significant decrease in plant length was observed under higher than 5g/L salinity stress, when the bacterial inoculation was unable to mitigate the effect of stress. Statistically significant decrease in plant total length was observed in the case of 5 g/L NaCl treated plants, when both bacterial strains inoculation caused a reduction in length (*Pantoea agglomerans* (8G/3) by 61%, whereas *Serratia fonticola* (BB17) by 54%). The maize plant total biomass values plotted in Fig. 5 show similar tendencies to those obtained for total length. A strong positive linear correlation was observed between the two datasets (Pearson correlation coefficient, r=0.72).

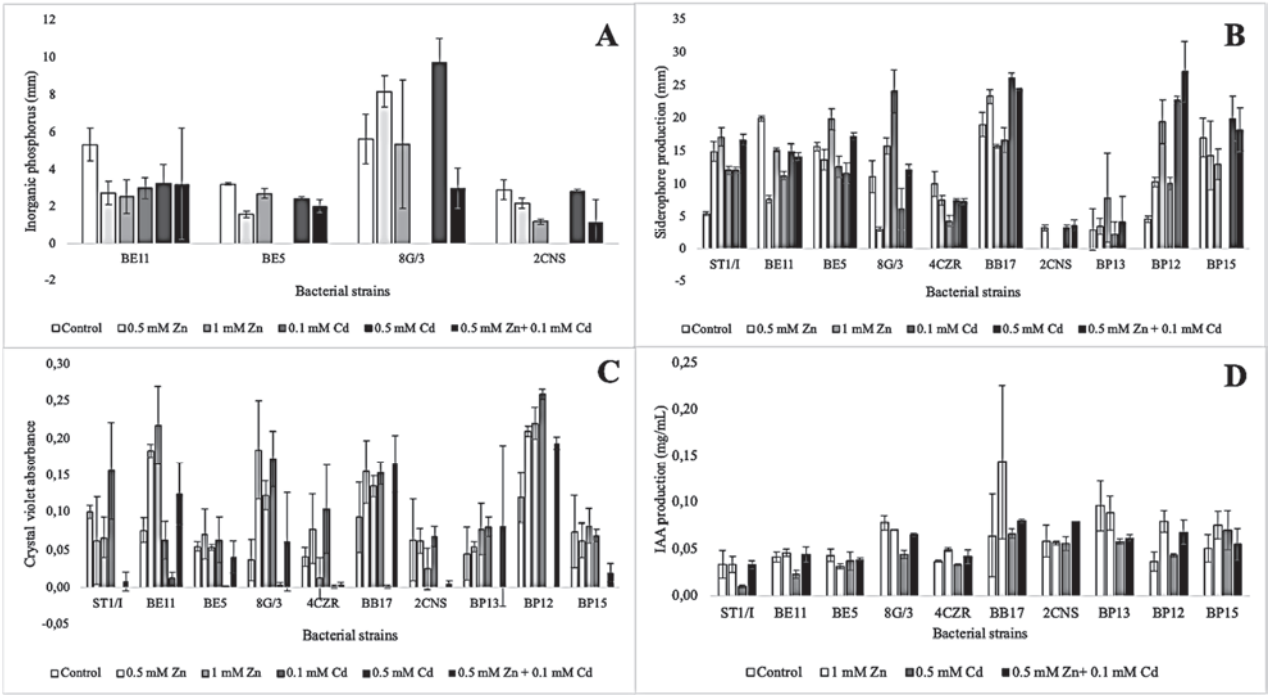

Fig. 1. Plant growth promoting traits of the selected bacterial strains in the presence of heavy metals. (A) inorganic phosphorus mobilization, (B) siderophore production, (C) EPS production (crystal violet absorbance), (D) IAA production

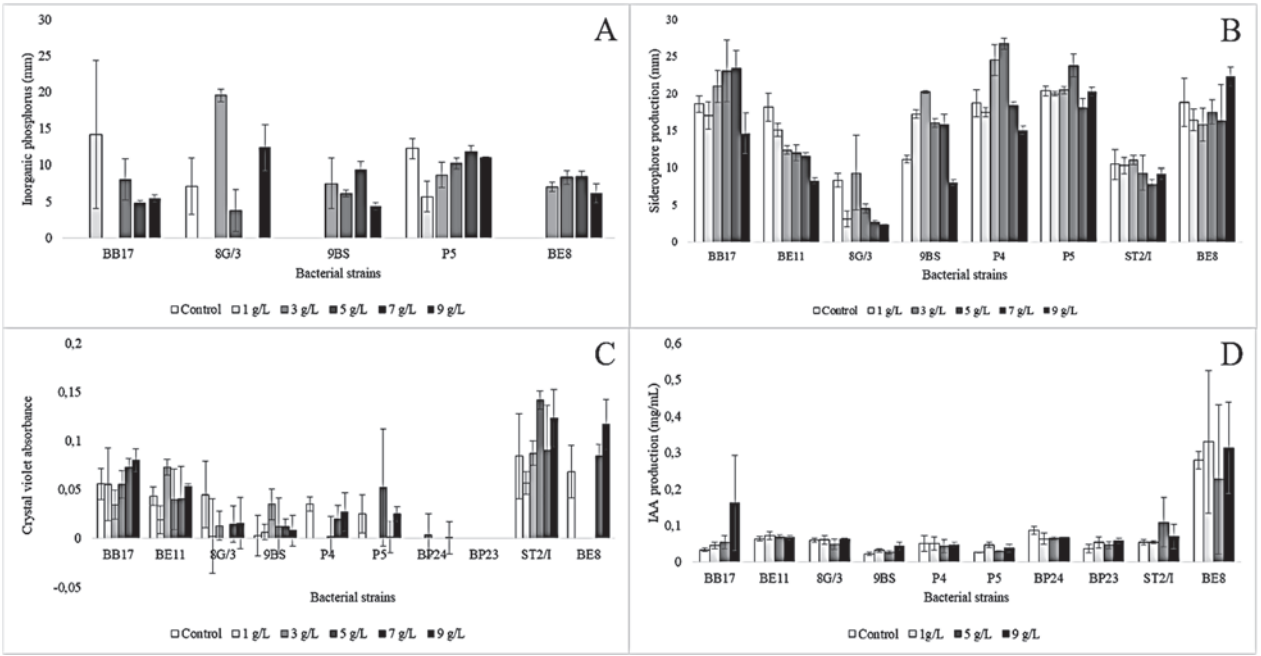

Fig. 2. Plant growth promoting traits of the selected bacterial strains in the presence of salt. (A) inorganic phosphorus mobilization, (B) siderophore production, (C) EPS production (crystal violet absorbance), (D) IAA production

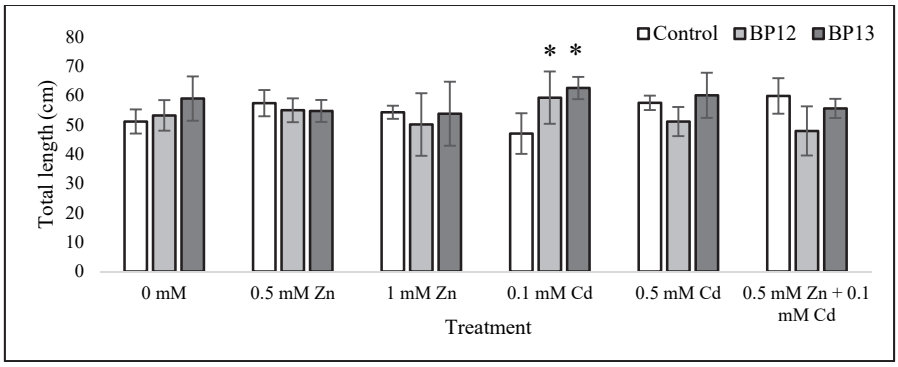

Fig. 3. Effect of heavy metal stress on the total length of the maize plants with and without PGPR inoculation. Altogether 7 plants were selected randomLy from each treatment. One-way ANOVA followed by Tukey test was computed to compare variables (mean±standard error). (∗ - significantly different from control at p<0.05 level)

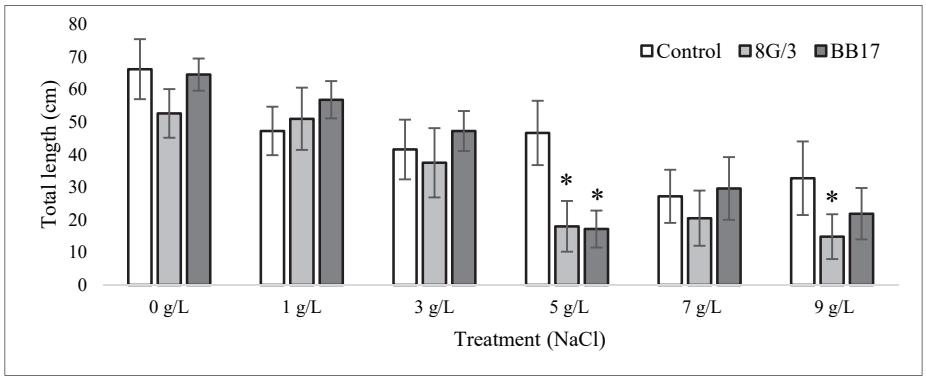

Fig. 4. Effect of salt stress on the total length of the maize plants with and without PGPR inoculation. Altogether 7 plants were selected randomLy from each treatment. One-way ANOVA followed by Tukey test was computed to compare variables (mean±standard error). (∗ - significantly different from control at p<0.05 level)

The biomass of maize plants treated with 1mM Zn and inoculated with *Delftia acidovorans* (BP12) (1.61±0.48) were significantly lower (29%, p=0.005) than the biomass of control plants (2.27±0.22). The growth of plants inoculated with both bacterial strains was promoted in the case of 0.1 mM cadmium treatment. *Delftia acidovorans* (BP12) statistically significantly enhanced the plant growth with 65.7% (p=0.031, whereas *Viridibacillus* sp. (BP13) increased it with 70.0% (p=0.0017).

The maize plant total biomass values represented in Fig. 6 strongly correlate with those obtained for total length (Pearson correlation coefficient, r=0.94). In case of lower salt concentrations an increase in plant total biomass with bacterial inoculation was observed (Fig. 4). *Serratia fonticola* (BB17) inoculation enhanced significantly the maize plant biomass with 57% (1 g/L NaCl, p=0.002) and with 37% (3 g/L NaCl, p=0.002). A statistically significant decrease in plant length was observed in higher than 5g/L salinity stress, where the bacterial inoculation was unable to alleviate the side effect of salt stress.

3.4. Effect of abiotic stress and bacterial inoculation on the chlorophyll content of maize plants

The total chlorophyll content of the heavy metal stressed maize plants with and without bacterial inoculation was determined (Fig. 7). In stress-free environment, the *Viridibacillus* sp. (BP13) bacterial strain enhanced the total chlorophyll content of the maize plant (59%, p=0.017). The *Delftia acidovorans* (BP12) bacterial inoculation had positive effect on plant growth when a 0.5 mM Zn, 1 mM Zn and 0.1 mM Cd treatment was applied, but the differences were statistically non-significant. The *Viridibacillus* sp. (BP13) inoculation was not able to diminish the effect of the 0.5 mM Zn and 0.5 mM Cd treatment, causing a reduced chlorophyll content in both cases (44% decrease in 0.5 mM Zn p=0.0025; and 38% decrease in 0.5 mM Cd, p=0.0014). The chlorophyll content of the plants under heavy metal stress showed weak positive correlation with length and biomass obtained for each treatment (Pearson correlation coefficient, r=0.34 in both cases).

The chlorophyll content of the maize plants was diminished in the presence of the salt stress whether bacterial inoculation was applied or not (Fig. 8). However, under no stress condition, the chlorophyll content of the plants was moderately enhanced by both PGPR strains, but the differences were statistically non-significant. When 1 g/L salt stress was applied, the *Serratia fonticola* (BB17) bacterial strain inoculation produced a statistically significant increase in chlorophyll content (56%, p=0.0009).

At higher salt concentration, the above-mentioned bacterial strain was unable to diminish the salt stress, whereas the *Pantoea agglomerans* (8G/3)

inoculation caused both negative and positive effects on the total chlorophyll content of maize plants. In case of 3 g/L salt stress a 36% decrease (p=0.01) whereas in case of 5 g/L salt stress a 46% increase (p=0.03) in chlorophyll content was obtained. Trends obtained for chlorophyll content change under salt stress condition when compared to length (moderate positive correlation, r=0.52) and biomass (weak positive correlation, r=0.44) obtained for each treatment. All four PGP bacterial strains increased the length, biomass and chlorophyll content of maize plants without abiotic stress conditions.

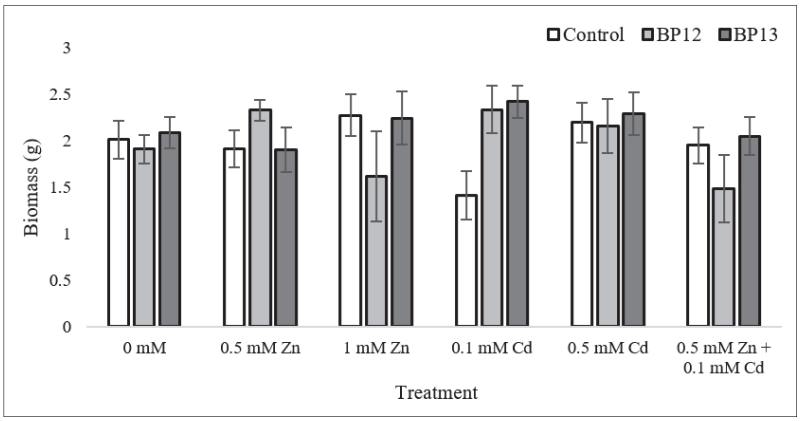

Fig. 5. Effect of heavy metal stress on the total biomass of the maize plants with and without PGPR inoculation. Altogether 7 plants were selected randomLy from each treatment. One-way ANOVA followed by Tukey test was computed to compare variables (mean±standard error). (∗ - significantly different from control at p<0.05 level)

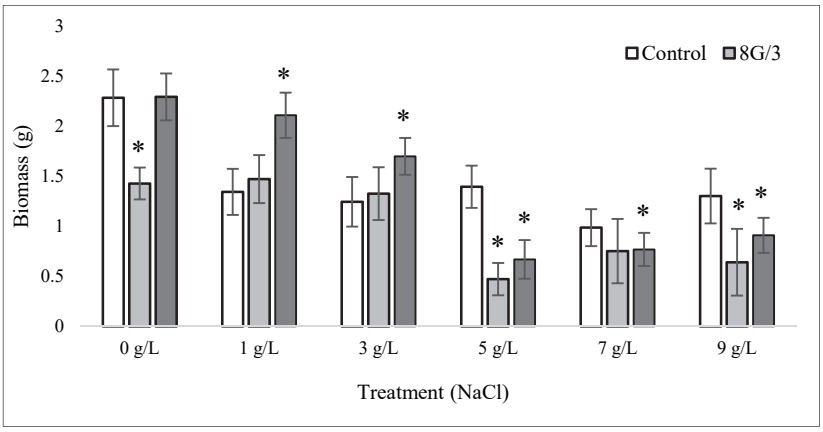

Fig. 6. Effect of salt stress on the total biomass of the maize plants with and without PGPR inoculation. Altogether 7 plants were selected randomLy from each treatment. One-way ANOVA followed by Tukey test was computed to compare variables (mean±standard error). (∗ - significantly different from control at p<0.05 level)

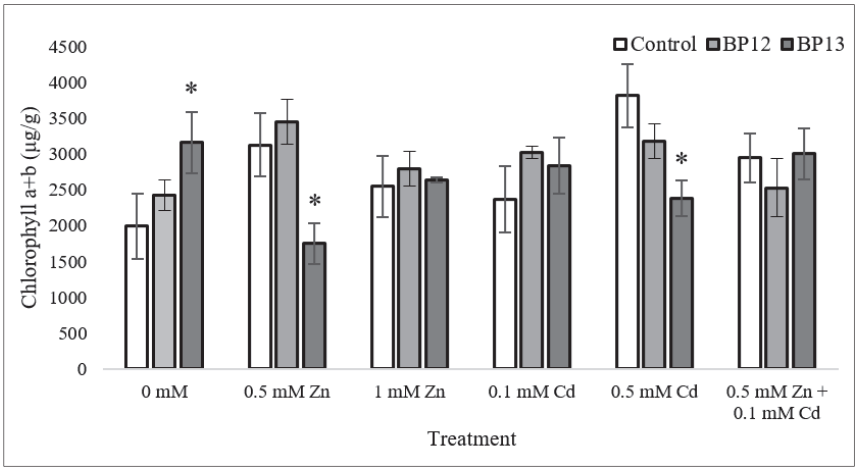

Fig. 7. Effect of heavy metal stress on chlorophyll content of the maize plants with and without PGPR inoculation. Altogether 3 plants were selected randomLy from each treatment. One-way ANOVA followed by Tukey test was computed to compare variables (mean±standard error). (∗ - significantly different from control at p<0.05 level)

When the abiotic stress was applied no clear evidence of beneficial effect of PGP bacterial inoculation on maize was observed. In case of 0.1 mM Cd treatment both bacterial strains (*Viridibacillus* sp. (BP13) and *Delftia acidovorans* (BP12)) increased the length and biomass of maize plants, mitigating the effect of heavy metal stress. The *Viridibacillus* sp. as potential microbial inoculant was previously described, being able to promote plant growth of maize, tea, pea and wheat plants, and to maintain PGP traits under abiotic stress conditions (acidity, desiccation and salinity) (Thakur et al., 2017). *Delftia* sp. bacterial strains were formerly mentioned in scientific literature as showing heavy metal resistance and also PGP traits (Braña et al., 2016). Liu et al. (2018) reported a *Delftia* sp. strain that reduced the cadmium accumulation in *Oryza sativa*. No plant growth promoting effect of above-mentioned bacterial strains under heavy metal stress conditions was previously described. The *Serratia fonticola* (BB17) bacterial strain inoculation at lower salt concentrations (until 3 g/L) increased the biomass and chlorophyll content of the maize plants, whereas at 5 g/L NaCl concentration *Pantoea agglomerans* (8G/3) inoculation caused an increase in total chlorophyll content. El-Esawi et al. (2018) described the positive effect of *Serratia liquefaciens* inoculation on the growth of maize plants under salt stress, and also the salt tolerance induction due to upregulation of the stress related genes. The beneficial plant growth promoting effect of *Pantoea agglomerans* was proved on maize plants under salt stress in laboratory and greenhouse experiments (Gond et al., 2015).

3.5. Effect of abiotic stress and bacterial inoculation on antioxidant enzyme activity of maize plants

Antioxidant enzymes as guaiacol peroxidase are important in reducing heavy metal (Štolfa et al., 2015) and salinity (de Azevedo Neto et al., 2006) induced oxidative damage, playing an important role in ROS scavenging processes. Guaiacol peroxidase specific activity showed a decreased value with the increase of abiotic stress without bacterial inoculation in both cases (Tables 1-2).

The bacterial inoculation usually increased the values of GPOX specific activity with the increase of abiotic stress, but not all differences were statistically significant. A statistically significant increase was produced in case of 0.5 mM Cd and 0.1 mM Zn+0.5 mM Cd treatment by the inoculation with *Viridibacillus* sp. (BP13). The *Pantoea agglomerans* (8G/3) bacterial strain increased statistically significant the GPOX activity when maize plants were subjected to higher salt stress (7 g/L and 9 g/L). In case of 9 g/L salinity stress the inoculation with *Serratia fonticola* (BB17) increased the GPOX specific activity significantly, compared to control. Štolfa et al. (2015) report that due to different conditions (heavy metal species and concentration) and plant species the GPOX activity varies, either increase or decrease were observed. In stress tolerance the induction of the antioxidative system is indispensable, therefore stress tolerant plants show an increase in antioxidant enzyme activity (de Azevedo Neto et al., 2006) that may be the result of the *de novo* protein synthesis or the activation of enzymes already present in plant cells to diminish ROS deleterious effects (Štolfa et al., 2015).

The present study thus revealed that the high GPOX activity under *Viridibacillus* sp. (BP13) bacterial inoculation in case of heavy metal stress, as well as *Pantoea agglomerans* (8G/3) and *Serratia fonticola* (BB17) inoculation in case of salt stress may confer maize plants a higher resistance to abiotic stress.

4. Conclusions

In the present study it was described how two heavy metal tolerant PGPR strains *Viridibacillus* sp. (BP13), *Defltia acidovorans* (BP12) as well as two salt tolerant strains *Pantoea agglomerans* (8G/3), *Serratia fonticola* (BB17) maintain their plant growth promoting traits under stress conditions.

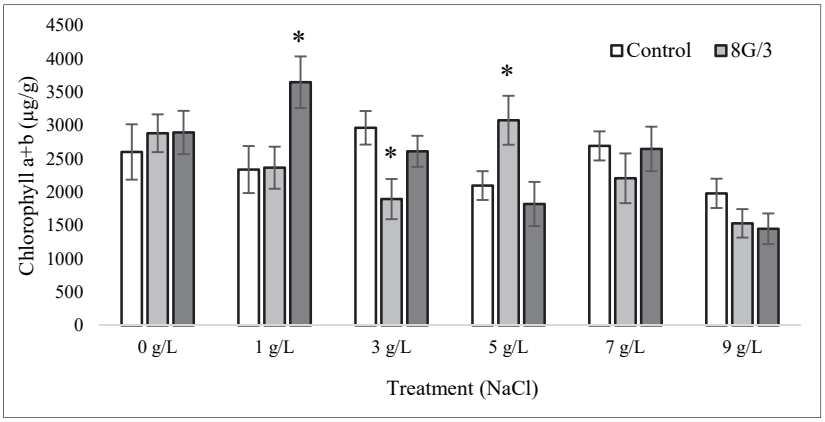

Fig. 8. Effect of salt stress on chlorophyll content of the maize plants with and without PGPR inoculation. Altogether 3 plants were selected randomLy from each treatment. One-way ANOVA followed by Tukey test was computed to compare variables (mean±standard error). (∗ - significantly different from control at $p<0.05$ level)

Table 1. Comparison of guaiacol peroxidase (GPOX) specific activity (U/mg) between *Zea mays* control plants and bacterial-treated plants under stress condition. One-way ANOVA followed by Tukey-test were used to compare variables. Statistically significant differences at $p>0.05$ were considered

Heavy metal stress	***Control plant***	***BP12***	***BP13***
GPOX specific activity in U/mg protein			
No stress	0.0049±0.0017	0.0041±0.0016 F=0.5096, p=0.4956	0.0078±0.0046 F=1.712, p=0.227
0.5 mM Zn	0.0060±0.0022	0.0055±0.0046 F=0.0489, p=0.8305	0.01169±0.0080 F=2.273, p=0.1701
1 mM Zn	0.0055±0.0031	0.0063±0.0049 F=0.0992, p=0.7608	0.0085±0.0025 F=2.676, p=0.1405
0.1 mM Cd	0.0039±0.0019	0.0059±0.0015 F=3.251, p=0.1091	0.0171±0.0101 F=8.106, **p=0.0215**
0.5 mM Cd	0.0035±0.0018	0.0044±0.0052 F=0.1563, p=0.7029	0.0088±0.0023 F=15.68, **p=0.0041**
0.5 mM Zn+0.1 mM Cd	0.0050±0.0025	0.0018±0.0016 F=5.664, **p=0.0445**	0.0120±0.0029 F=16.18, **p=0.0038**

Table 2. Comparison of guaiacol peroxidase (GPOX) specific activity (U/mg) between *Zea mays* control plants and bacterial-treated plants under stress condition. One-way ANOVA followed by Tukey-test were used to compare variables. Statistically significant differences at $p>0.05$ were considered

Salt stress	***Control plant***	***8G/3***	***BB17***
GPOX specific activity in U/mg protein			
No stress	0.0089±0.0028	0.0070±0.0019 F=1.506, p=0.2546	0.0066±0.0011 F=2.586, p=0.1465
1 g/L NaCl	0.0092±0.0025	0.0073±0.0018 F=1.915, p=0.2038	0.0045±0.0030 F=6.957, **p=0.0298**
3 g/L NaCl	0.0049±0.0011	0.0046±0.0037 F=0.0334, p=0.8594	0.0053±0.0026 F=0.1196, p=0.7384
5 g/L NaCl	0.0074±0.0028	0.0057±0.0028 F=0.0214, p=0.8885	0.0103±0.0058 F=1.042, p=0.3373
7 g/L NaCl	0.0062±0.0038	0.0176±0.0051 F=15.82, **p=0.0040**	0.0051±0.0034 F=0.2022, p=0.6648
9 g/L NaCl	0.0015±0.0014	0.0063±0.0038 F=6.609, **p=0.0331**	0.0081±0.0028 F=21.47, **p=0.0016**

The effect of abiotic stress resistant PGP bacterial strains was tested on maize plant growth and development. Both heavy metal resistant bacterial strains increased the length, biomass and chlorophyll content of the maize plants in the lack of heavy metal treatment. When heavy metal stress was applied no clear evidence of beneficial effect of PGP bacterial inoculation on maize plant growth and development was found in any treatment. In the case of 0.1 mM Cd treatment both bacterial strains increased the length and biomass of the maize, mitigating the effect of heavy metal stress. Both salt resistant bacterial strains increased the length, biomass and chlorophyll content of maize plants in the lack of salt stress. In the case of the applied salt concentrations, the beneficial effect of PGP bacterial inoculation on maize plant growth and development was confirmed only sporadically. With increasing salt concentration, a decrease in maize plant length was observed, regardless of the bacterial inoculation. The inoculation with *Serratia fonticola* (BB17) bacterial strain at lower salt concentrations (until 3 g/L) increased the biomass and chlorophyll content of the maize plants, whereas at 5 g/L NaCl concentration the inoculation with *Pantoea agglomerans* (8G/3) caused an increase in total chlorophyll content.

Despite the above mentioned sporadically observed beneficial effect of the PGP bacterial inoculation on growth and development, the higher GPOX activity observed during *Viridibacillus* sp. (BP13) bacterial inoculation in case of heavy metal stress, as well as the inoculation with *Pantoea agglomerans* (8G/3) and *Serratia fonticola* (BB17) in case of salt stress reveals that PGP bacterial strains increase the plant tolerance to abiotic stressors.

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