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Growth enhancement of maize by the use of selenium resistant bacteria under selenium stress

Abstract

Purpose: Heavy metal accumulation in environment is potentially creating problems to life especially to plants. Two selenium resistant bacteria, *Bacillus pumils* strain CrK08 and *Bacillus licheniformis* strain AsK03 were checked for their role in growth promotion of *Zea mays* in pot experiment under selenium stress.

Methods: The two bacterial strains *Bacillus pumils* strain CrK08 and *Bacillus licheniformis* strain AsK03 were assessed for their ability to promote plant in pot experiment and growth parameters were measured.

Results: Both strains significantly promoted root length (13%) in control and (51%) in autoclaved soil. Number of roots was reduced to 28% and 35% in control and autoclave soil respectively. Fresh weight and dry weight were reduced up to 2%, 1% and 28% and 25% in control and autoclave soil plants. In treated plants fresh weight was 35% and 16 % high than non-treated plants. Strains also produced an increase in soluble protein content. Indole acetic acid (IAA) content was enhanced to 10% and 65% while acid phosphatase activity was 13.3% and 50% lower in inoculated plants than respective controls. Peroxidase content reduced up to 2%. Selenium content in control plants was high as compared to treated plants.

Conclusion: On the whole these strains promote plant growth under Se stress (17mg/kg) in soil.

Keywords: selenium resistant bacteria, heavy metal, *Zea mays*, biochemical parameters, selenium content in plant

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Introduction

Human activities have posed deleterious effects on the natural resources especially land and water resources. Human activities have introduced a large number and huge quantities of dangerous chemicals like fertilizers, heavy metals and their salts, pesticides, chlorinated solvents and acids in to soil, water and air.[1] Each of these chemicals has its own detrimental effect on life, but the presence of heavy metals in natural resources are of special concern due to their multidimensional damaging activity e.g. carcinogenicity and mutagenicity.[2,3] Plants fulfill most of their metal input needs by process of atmospheric deposition. Selenium is an important essential element which is needed in trace quantities for plants, animals and humans. But selenium is also a heavy metal; its excessive exposure can cause environmental toxicity, water pollution and a number of problems like deformities and even death to larger animals.[4] Selenium is an important metabolite for life in minute quantities. It acts as co-factor in function of many critical enzymes. Rotruck found that selenium is a necessary part of glutathione peroxidase (GSH-Px) enzyme. Depending upon its concentrations, selenium has dual role i.e.it enhances plant growth by acting as an antioxidant in low quantities, and it reduces plant yield when present in high concentration.[5] Moreover, Se reduces the photo oxidative stress and activates defense mechanisms in potato chloroplasts.[6] Electro microscopic studies revealed that the growth of lettuce plant increases and higher quantities of starch accumulated when lettuce plants were provided with selenium doses.[7] Studies have also shown that Selenium prevents the photosynthetic apparatus of maize plants from oxidative damage by elevating levels of anti-oxidants.[8] Selenium enhances the plant growth by promoting accumulation of starch in chloroplast.[7]

Many Prototrophic and chemotrophic bacteria have the ability to reduce oxyanions of selenium Se (IV) and Se (VI), either to volatile form Se (II) or to elemental selenium Se (0).[9,10] Bacteria have adopted strategies to cope with the heavy metal stress. A number of mechanisms have been discovered through which bacteria tolerate heavy metal accumulation in the environment. Some of the important mechanisms for heavy metal resistance are reduction to less toxic form, efflux of metal ions, accumulation and complex formation inside the cell.[11] Free living bacteria that are useful for growth of plants by either providing beneficial nutrients to plants or by protecting from toxic chemicals are called plant growth promoting rhizobacteria (PGPRs), which are found in roots of many plants as symbionts.[12] PGPRs may enhance plant growth in two different ways; direct or indirect.[13] Direct growth promotion is through the production of certain growth promoting compounds by the bacteria or by increasing the nutrient intake by plants from the soil, while indirect growth promotion is either through decreased or complete prevention of deleterious effects of phyto pathogenic organisms. They may fix atmospheric nitrogen; produce siderophores; produce number of plant hormones including auxins and cytokinins; or may have mechanisms to solubilize minerals like phosphorus; and synthesis of IAA, hydrogen cyanide and ammonia.[14] Indirect development also involves enzymes that can amend plant growth and development.[13] So due to these reasons when seeds treated with PGPRs or the PGPRs are applied indirectly in to the soil they increase plant growth and yield and decrease heavy metal soil toxicity[15] and increase plant growth and yield.[13] In this study pot experimentation isdone in green house to assess the growth promoting effect of *Bacillus pumilus and Bacillus licheniformis* on *Zea mays* under selenium stress conditions and growth and biochemical parameters are determined. Selenium content

is also checked in plants and soil to glimpse the effect of bacterial inoculums on selenium accumulation by plants.

Materials and methods

Plant-microbe interaction study

Pot experiment was conducted to study plant - microbe interaction. Pre isolated bacterial strains, *Bacillus pumilus* - CrK08 and *Bacillus licheniformis* - AsK03 were used for the experiment. These strains were isolated from the soil polluted with tannery wastes from Kasur, Pakistan. Both strains were heavy metal resistant. CrK08 is Chromium resistant while ask03 is Arsenic resistant. Both strains are also selenium resistant and can resist up to 1000 µg ml^{-1} of selenium. The experiment was performed to check the plant growth under metal stress in presence of bacterial inoculums. The experiment was done under natural day light and temperature. Mixed bacterial cultures were directly inoculated into soil.

Experimental soil

The 30-50 cm layer of garden soil was collected. The soil was a loamy clay soil with a pH of 8. 2. The collected soil was allowed to dry at room temperature and then sieved through 2mm sieve and stored in plastic bags. Chemical characteristics of soil are given in Table 1.

Table 1 Soil characteristics

Ingredient	Amount
Sodium	5.43 g kg^{-1}
Potassium	0.12 g kg^{-1}
Calcium	2.16 g kg^{-1}
Magnesium	0.57 g kg^{-1}
Sulfate	2.8 g kg^{-1}
Carbonate	Nil
Bicarbonate	1.22 g kg^{-1}
Organic matter	3.61%
Phosphate	46.0 g kg^{-1}
Arsenic	Nil
Chromium	Nil
Selenium	50 µg g^{-1}
pH	8.2

Pot experiment

About 3 kg of soil was filled in each PVC pot (15x17cm). For control, pots were filled with soil without autoclaving. For soil autoclaving, the soil first weighed and packed in plastic bags and autoclaved at 12°C for 15 minutes at 15atm pressure. The soil was allowed to cool at room temperature for 24 hours after that a second autoclaving was done and again cooled to room temperature. Pots were filled with soil and arranged in setup as shown in Table 2.

Table 2 Experimental setup

	Control soil		Autoclaved soil	
Bac	Control P1	P1	Control P2	P2
AsK03+CrK08	Without Inoculum	AsK03+CrK08	Without Inoculum	AsK03+CrK08

Control P1, control plant of non-autoclaved/control soil; P1, Control soil with bacterial inoculums; Control P2, autoclaved control; P2, autoclaved soil with bacterial inoculums

Seeds Sterilization

Seeds of var. NK-6326 by Sygenta (pvt) Ltd. obtained from National Agriculture Research Center (NARC), Islamabad, Pakistan were sterilized with 0.1% $HgCl_2$ for 6 minutes and then washed five times with autoclaved distilled before sowing. Seeds were transferred to autoclaved petri plates. Seed viability was also checked by growing them on filter paper.

Experimental setup

Soil was brought to water capacity before sowing. Sterilized seeds were planted 1cm deep in the soil. The pots were arranged in lanes in net house at an average daily temperature. Seed emergence process was observed daily. Initially 12 seeds per plant for maize were sown. After complete germination thinning was done to 9 plants per pot. For bacterial culture preparation, bacteria were grown in L-Broth at 37ºC overnight. The cultures were harvested, washed and re-suspended in sterilized distilled water. The final bacterial cell density was adjusted to 10^8 CFU/ml. Bacterial mixed cultures were prepared by mixing equal quantity of single (pure) culture. After thinning, the mixture was directly poured in the respected pots. 50ml of metal solution containing 100 µg ml^{-1} of Na_2SeO_3 was applied to all pots of each bacterial as well as control treatment. With a gap of three days, the pots were again watered with 50 ml of metal solution. Three replicas of each treatment were carried out. Pots were watered at regular basis with measured quantity and allowed to grow. Plant growth and development parameters was recorded throughout the growing season. After four months plants were carefully removed from pots and washed with distilled water to remove soil and other debris.

Growth parameters

Various growth parameters of plant growth were considered i.e. Shoot length (cm), Root length (cm), No. of leaves, No. of roots, Fresh weight of plant (g), Dry weight per gram of fresh weight of plant (mg /g), Dry weight of plant (g).

Biochemical parameters

By performing the Mahadevan[16] protocol, auxins were extracted from shoots of plants. Soluble proteins were extracted following method of Bhatti et al.,[17] while soluble proteins were analyzed by the method of Lowry et al.,[18] For the estimation of peroxidases quantity David and Murray method was used. Iqbal[19] method were used for the acid phosphatases extraction.

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Estimation of selenium content

The Humphries (1956) method was used for the estimation of selenium content in plants. The plants were first dried in oven at 80°C for 24 hours, then the plants were removed and dried plants were cut into small pieces. Properly washed and dried conical flasks were taken and labeled according to the treatment. Pieces of weighed plant material were taken in the respective flasks. 10ml conc. Of HNO_3 and 2ml of $HClO_4$ was added in each flask. Plant material digestion was done by heating on the water bath. Digested material was diluted up to 10ml with distilled water and the selenium contents was analyzed with the help of Beckman D2 Spectrophotometer.

Modified Watkinson[20] method was used to determine selenite contents which involve use of spectrophotometer. First,10 ml of 0.1M HCl, 0.5 ml of 0.1M EDTA, 0.5 ml of 0.1M sodium fluoride, and 0.5 ml of 0.1M disodium oxalate were mixed in test tube. 250µl sample was added to the tube and then 2.5 ml of 0.1% 2, 3-diaminonaphthalenein and 0.1M HCl was added to it. After thorough mixing the tubes were incubated at 40°C for 30 minutes to form selenium 2, 3-diaminonaphthalene complex and then allowed to cooled down at room temperature. 6ml of cyclohexane was used to extract the selenium 2, 3-diaminonaphthalene complex by shaking the tubes vigorously for about 1 minute. The absorbance of organic phase was observed at 377 nm. Calibration curve was made by making solutions of known concentrations of sodium selenite in distilled water.

Results

Soil characteristics

Soil was analyzed and different soil contents were recorded. This analysis includes soil pH, organic matter, metal contents like sodium, potassium, calcium, magnesium, arsenic chromium and selenium etc. All the results of analysis are given in the Table 1.

Impact of metal stress on *Zea mays*

Plant population is one of the most critical factors in determining the crop yield and this also depends upon seed viability. So, before performing experiment in the field seed viability was checked by growing them in petri plates. Seeds have good viability as seed germination occurred after 24 hours. Slight effects of metal toxicity on plant appearance and growth was observed as the lower leaves of all plants dried soon. Purpling of leaves was also observed in all plant of control as well as autoclaved soil that may be because of phosphorus deficiency. More purple color was observed in plants with control soil as compared to autoclaved soil. Plants in the autoclaved soil looked healthier than the plants in control soil.

Harvesting of plants

The plants were observed for enough proper time as the traditional crops and were harvested after 21 weeks.

Growth parameters

There were actually four types of plants that were grown. Control plants of non-autoclaved soil were named as P1, Control soil with bacterial inoculums was P2, autoclaved control P3 and autoclaved soil with bacterial inoculums was given the name P4. Seed germination started at 5th day after sowing and continued for five days. Percentage seed germination was 80.5 % in control (non-autoclaved) soil while 87.5 % in autoclaved soil. An increase of 7 % in seed germination was observed in autoclaved soil. There was an increase of 13% seed germination in control plants P2 having the bacterial inoculum. A significant increase in root length was observed of inoculated plants P2 and P4. P4 showed an extra increase 51% increase in root length as compared to P2. A decrease of 34.9% in root length was observed in control P3 while no significant decrease in P1. As far as shoot length is concerned mix results were obtained as P2 plants on control soil showed an increase in shoot length as compared to un-inoculated P1 plants. In autoclaved soil there was a decrease in shoot length in P4 inoculated as compared to un-inoculated P3 plants. Fresh weights of all inoculated plants were decreased with respect to control plants. There was a slight decrease in fresh weight of P2. Similarly, in autoclaved soil decrease in fresh weight was observed in P4 but the difference was not very significant. In case of dry weight, increase in dry weight took place in plants of autoclaved soil when compared with control soil. In control and autoclaved soil, a decrease in dry weight was observed when control plants were compared with inoculated one. The inoculated plants of both control and autoclaved soils showed an increase in dry weight as compared to their un-inoculated controls.

Biochemical parameters

There was a decrease in auxin content (10%) in inoculated plant P2 as compared to non-autoclaved soilcontrol plant P1 while a marked increase (65%) in inoculated plants P4 as compared to control plants P3 of autoclaved soil. An increase of 9% in protein content was found in inoculated plants of control as well as autoclaved soil when compared to their respective controls. There was an increase in peroxidase activity (0.27%) in inoculated plants of control soil as compared to control plant of this soil while 2% decrease was observed in case of autoclaved soil in which enzyme activity decreased in inoculated plants as compared to control. Acid phosphate activity was 13.3% and 50% less in inoculated plants as compared to controls both in non-autoclaved and autoclaved soil. Selenium contents were maximum in control plants P1 of control soil and were minimum in inoculated plants P4 of autoclaved soil. A significant decrease of up to 50% was observed in inoculated plants in both control and autoclaved soil. Metal content was high in soils of inoculated pots 8% and 1% high as compared to their controls. Metal content in soil in soil analysis was also determined before and after harvesting of plants. There was a marked decrease in the amount of selenium in the soil of all treatments when compared with the initial metal content in soil after the addition of metal. Minimum amount of metal was present in control plant P1 and P3 while the highest metal content was found in soil of autoclaved soil P4 plants in which inoculums was given.

Discussion

Bacteria play vital role in determining the physical and chemical characteristics of soil and soil quality.[21] In current study bacterial strains used to inoculate *Zea mays* var. NK 6326 significantly affect plant growth. Plant growth promoting rhizobacteria (PGPRs) can affect the plant growth in two ways either to indirectly protect the plant from metal toxicity or to directly produce the substances that enhance plant growth.[22] In case of fresh weight, dry weight and dry weight per gram of fresh weight, increase in biomass was observed in case of autoclaved soil. It may be due to absence of antagonistic effect of other microorganisms in the soil. The sterilized soil induces bias since inoculants are not in competition with resident microorganisms of soil and protozoa. Biro et al.,[23] also observed such results under similar conditions. ACC deaminase synthesized by many bacterial species like *Pseudomonas brassicacearum*, *P.marginalis*, *P. oryzihabitans*, *Bacilluspumilus* and *Rhodococcus* have been shown to increase the plant biomass but not the rate of accumulation of metals

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by plants.[24] Hartikainen showed the growth enhancement effect of bacteria in presence of selenium on Ryegrass. Less metal absorption in plants is characterized by the absorption of metal by inoculant microorganism.[25] In maize decrease concentration of selenium in plants indicate bisorption of metal by *Bacillus pumilus* and *Bacillus licheniformis* as compare to soil without inoculums where metal accumulated by plants is 100 times higher than without inoculums. Tables 3&4.

Table 3 Effect of selenium resistant bacteria on root and shoot length, fresh weight, dry weight and fresh weight per gram of dry weight of *Zea mays*

Strains	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Dry weight /fresh weight (mg/g)
P1	71.7	24.9	26	4.3	0.17
P2	82.5	27.4	23.74	3.36	0.16
P3	53.12	29	24.58	6.29	0.26
P4	108	24.9	23.3	5.04	0.19

P1, control plant of non-autoclaved/control soil; P2, Control soil with bacterial inoculums; P3, autoclaved control; P4, autoclaved soil with bacterial inoculums

Table 4 Effect of selenium resistant bacteria on growth parameters of *Zea mays*

Strains	Auxin (µg ml^{-1})	Soluble protein (µg ml^{-1})	Peroxidase (unit/g)	Acid phosphatase(K.A unit/100ml)
Cont P1	25	63	3.67	834.4
P1	22.8	71	5.03	736
Cont P2	24.8	60	5.64	1066.2
P2	70.8	69	3.66	635.2

Cont P1, control plant of non-autoclaved/control soil; P1, Control soil wit bacterial inoculums; cont P2, autoclaved control; P2, autoclaved soil with bacterial inoculums

The strains used in the study have the tendency to enhance the growth of plant in the presence of selenium. The present strains significantly increase auxin content of plant facilitating its shoot elongation and when used in autoclaved soil it has the ability to promote root length. Increase in soluble protein act as a dilution factor for metal in plants that is enhanced in plants in the presence of metal and inoculums. In inoculated plants increase in protein content may be due to increase in nutrient availability and nitrogen fixation. The inoculation with plant growth promoting bacteria improve seed nitrogen, protein content and phosphorus content of *salicornia* species of sunflower. Aon et al.,[26] showed that enzymes have strong correlation and their activity play a very important role between physical, chemical and microbial soil properties which ultimate enhance plant growth.[27]

Conclusion

It is concluded from above discussion that *Bacillus pumilus* and *Bacillus licheniformis* have ability to promote growth of plants in various ways i.e. increase in root length, shoot length, and plant height. It also enhances the production of growth hormones like auxin and many other biochemical parameters like soluble protein and peroxidase activity).Hence the strains can be used to help the plants to growth in the soil polluted with selenium (up to 17mg/ kg). Further the plants can be used as selenium supplements to treat selenium deficiency in animal diet etc.

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None.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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