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Full Length Article

Effect of *Bacillus fortis* 162 on Growth, Oxidative Stress Tolerance and Phytoremediation Potential of *Catharanthus roseus* under Chromium Stress

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Abstract

The present research was performed to evaluate the role of *Bacillus fortis* 162 on growth, chromium (Cr) uptake and subsequent oxidative stress tolerance of *Catharanthus roseus* grown in 0, 50, 100 and 200 mg Cr kg^{-1} soil. The extent of oxidative stress was assessed by observing the activities of antioxidant enzymes, along with quantification of malondealdehyde (MDA), hydrogen peroxide (H_2O_2) and proline contents of treated plants. The *B. fortis* 162 enhanced shoot length, root length and biomass production of inoculated plants as compared to un-inoculated ones. The plants growing in higher concentration of Cr exhibited augmented Cr contents as well as higher level of MDA and H_2O_2. The Cr stress tolerance was attributed to the improved activities of antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and reduced level of MDA and H_2O_2 in inoculated *C. roseus* plants. The growth promoting traits of *B. fortis* 162 such as 1-aminocyclopropane-1-carboxylate deaminase (ACCD) activity, phosphate solubilization, siderophore and auxin production improved growth and Cr uptake in treated *C. roseus* plants. The present research offers novel information about growth promotion potential of *B. fortis* 162 in *C. roseus* by decreasing Cr induced oxidative stress. © 2018 Friends Science Publishers

Keywords: *Bacillus fortis*; Chromium; Phytoremediation; Stress

Introduction

Many industrial effluents and solid wastes of Pakistan and other countries contain heavy metals which pollute our environment and food chain. Heavy metal toxicity cause abiotic stress in plants growing in affected areas (Gamalero and Glick, 2012). Metal toxicity and other kinds of stress impede growth and metabolic activities of crops (Khan *et al*., 2016a). Among these toxic metals, chromium (Cr) is an important pollutant and is extremely harmful to different ecosystems (Abdullah *et al*., 2015). Chromium is a steel-gray heavy metal which is probably the 6th major element on the earth's crust. It usually exists as hexavalent Cr (VI) or trivalent Cr (III) form. This metal is highly resistant to oxidation and may cause gastrointestinal problem, mutation and cancer in living organisms (Costa, 2000). Chromium is extensively used in metallurgical, chemical and tannery industry. The continuous disposal of industrial wastes containing Cr is increasing surface and subsurface pollution in Pakistan (Rashid *et al.,* 2012). This alarming situation necessitates removal of Cr by adopting different physicochemical techniques. The infrastructure of these techniques is usually very expensive. Furthermore, these techniques may cause secondary pollution and deteriorate soil health and fertility (Mosa *et al*., 2016). It is, therefore, necessary to find some environment friendly and economic methods for decontamination of Cr and other heavy metals (Bah *et al*., 2010).

Bioremediation have a significant role in reclamation of heavy metal affected sites (Kamran *et al.,* 2015). Some plants have an activated chain of defense system to withstand toxicity of metals and may accumulate higher quantity of these metals in their root and shoot (Antonkiewicz *et al.,* 2016). Researchers are evaluating potential of phytoremediation technique for removal of Cr (Liu *et al*., 2011). The agronomic crops such as maize and *Crotalaria juncea* may accumulate a considerable amount of Cr (Prajapati *et al.,* 2012). Similarly a few ornamental crops including tube rose, *Nerium* spp. and *Jasminum* spp. are also capable to accumulate Cr (Santiago and Santhamani, 2010). The removal of Cr by growing hyper-accumulator plants is a safe and sustainable technique (Gołda and Korzeniowska, 2016). However, severe metal toxicity causes oxidative stress in plants (Golshan *et al.,* 2011). The increased levels of reactive oxygen species (ROS) limit cultivation and phytoextraction potential of hyper-accumulators under Cr stress. The survival of plants depends upon the equilibrium between formation and

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suppression of ROS (Khatun *et al.,* 2008). The antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) help plants to reduce this oxidative damage. Numerous plant species assisted with beneficial microbes help to eradicate environmental pollution (Kamran *et al*., 2014). Some rhizospheric bacteria also help in stress alleviation which in return increases growth and metal uptake in associated plants (Egamberdieva *et al.,* 2010). The enzymatic activities exhibited by rhizospheric microbes improve growth and stress tolerance of plants when growing in heavy metal contaminated media (Yousaf *et al*., 2010). These microbes act as soil conditioner and improve soil structure and chelate heavy metal enabling plants to uptake these chelated ions (Andrades-Moreno *et al.,* 2014). Metal resistant plant growth promoting rhizobacteria (PGPR) may help plants to mitigate stress of that particular metal. A few strains of *Bacillus spp.* may tolerate heavy metal and make it bioavailable to associated plants. That's why researchers tend to inoculate plants with *Bacillus spp.* to enhance growth and phytoremediation potential in heavy metal contaminated soil (Lu and Zhang, 2014).

In our previous research, it was observed that *B. fortis* 162 may improve phytoextraction and stress alleviation of *Althea rosea* plants under nickel stress (Khan *et al*., 2016b). Keeping in view the significance of *B. fortis* 162 assisted phytoremediation, *C. roseus* which is a fast growing cosmopolitan ornamental plant was grown in association with *B. fortis* 162. The core purpose of this study was to analyze the potential of *B. fortis* 162 in alleviation of Cr induced stress and phytoextraction capability of *C. roseus*.

Materials and Methods

Collection and Analysis of Soil Samples

The physicochemical properties of the soil samples collected (up to 30 cm) from Conventional and Non-conventional Farm of the University of the Punjab, Lahore Pakistan were estimated according to Amna *et al*. (2015). Afterwards, soil samples were amended with $K_2Cr_2O_7$ at the rate of 50 (C1), 100 (C2) and 200 (C3) mg Cr kg^{-1} soil, filled in sterile plastic pots (6" × 5") and placed at 23±4°C in shade for two weeks. Water holding capacity of the incubated soil samples was maintained at 55% by adding sterile distilled water.

Evaluation of Cr Tolerance

B. fortis 162 obtained from Bacterial Conservatory, University of the Punjab Lahore, Pakistan was cultured in LB broth medium for 2 days. The bacterial cells isolated by centrifugation at 4000 rpm for 15 min were re-suspended in distilled sterilized water and bacterial concentration was adjusted to 1×10^8 CFU mL^{-1} with the help of spectrophotometer (OD 1.0 at 600 nm). The Cr tolerance of microbe was evaluated by inoculating 10 μL of bacterial suspension on LB agar plates contaminated with different concentration of Cr viz.: 50, 100, 200, 300 and 400 mg L^{-1} at 28°C. After five days of incubation, the concentration above which bacteria couldn't grow was deemed as threshold level of Cr resistance. In case of positive control plates without Cr contamination, the diameter of bacterial colonies was approximately 4 mm.

Growth Promoting Characteristics of *B. fortis* 162

To evaluate auxin production, *B. fortis* 162 was cultured for 24 h in LB media containing 500 μg mL^{-1} L-tryptophan. An aliquot (1 mL) of this supernatant was mixed with 50 μL of 10 m*M* orthophosphoric acid and 2 mL of reagent (0.5 mL of 0.5 M $FeCl_3$ in 50 mL of 35% $HClO_4$). After 0.5 h of incubation, the absorbance of developed pink color was measured at 530 nm. The auxin concentration in culture was determined with the help of a calibration curve of pure auxin as a standard (Bric *et al*., 1991).

B. fortis 162 was cultured in Pikovskaya's medium containing 0.5% of tricalcium phosphate and production of clear zones was recorded as positive result for phosphate solubilization capability (Sundara-Rao and Sinha, 1963). The siderophore was determined by measuring diameter of the orange halo formed in chrome azurol S agar media (Schwyn and Neilands, 1987).

The ACCD activity of *B. fortis* 162 metabolites was estimated by quantification of α-ketobutylate synthesized during enzymatic hydrolysis of 1-aminocyclopropane-1-carboxylate (ACC) (Belimov *et al.,* 2005) and measuring absorbance at 540 nm and subsequent comparison with a standard curve of α-ketobutylate (Honma and Shimomura, 1978).

Inoculation of *B. fortis* 162

The bacterial inoculum (4 mL) was injected and mixed thoroughly in the soil media (Hadi and Bano, 2010). In case of un-inoculated control 4 mL distilled water was added in the soil. The surface sterilized seeds of *C. roseus* obtained from Sunny View Seed importers, Lahore Pakistan were sown individually in plastic pots. Five *C. roseus* seeds were sown at equidistance in each pot. All pots were placed in green house at 23±5°C.

Analysis of Proline and Chlorophyll Content

After 60 days, plants were harvested. Proline contents from leaves sample were measured with the help of spectrophotometer at 520 nm (Bates *et al*., 1973). Chlorophyll contents of leaves samples were analyzed with the help of chlorophyll meter (Model SPAD-502 Singapore) (Nazir, 2013).

Assay of Antioxidant Enzymes

For preparation of enzyme extract, 500 mg leaf tissue was

ground in 3 mL of 50 n*M* tris-HCl (pH=7.5) buffer containing 3 m*M* $MgCl_2$, 1 mM EDTA, with the help of pre-cooled mortar and pestle. The resulting mixture was centrifuged at 5000 rpm for 20 min at 4˚C.

Superoxide dismutase (SOD) was estimated according to Giannopolitis and Ries (1977). The 3 mL reaction mixture containing 63 µ*M* nitroblue tetrazolium, 1.3 µ*M* riboflavin, 13 m*M* methionine, 0.1 m*M* EDTA, 50 m*M* Tris–HCl (pH 8.0), and 50 μL extract was kept under light. After 20 min absorbance was recorded at 560 nm. The amount of enzyme required to inhibit 50% of the nitroblue tetrazolium photoreduction, in comparison with tubes lacking the plant extract was termed as one unit of SOD.

Peroxidase was evaluated by method described by Upadhyaya *et al.* (1985). The reaction mixture used contained 1 mL of 1% H_2O_2, 2.5 mL of 50 m*M* phosphate buffer (pH 6.1), 20 μL of enzyme extract and 1 mL of 1% guaiacol. The guaiacol oxidation rate was determined at 420 nm.

Catalase was determined at 25°C. For this purpose, reaction mixture included 2.5 mL of 50 m*M* phosphate buffer (pH= 7.0) with 0.2 mL hydrogen peroxide (1%) and 0.3 mL enzyme extract. The CAT was measured by monitoring a decrease in absorbance of H_2O_2 using an extinction coefficient 0.0436 (m*M* cm) at 240 nm (Aebi, 1984).

Analysis of Hydrogen Peroxide and Lipid Peroxidation

Hydrogen peroxide contents were analyzed in accordance with Velikova *et al.* (2000). Whereas, lipid peroxidation level in leaf samples of treated plants was determined in terms of malondialdehyde (MDA) content (Rao and Sresty, 2000).

Analysis of Growth Parameters and Cr Content

The harvested plants were washed thoroughly with sterilized water. Root and shoot samples were separated and dried in oven at 80℃. The pre weighted plants samples were digested in HNO_3.$HClO_4$ solution. The Cr content in digested samples were analyzed by atomic absorption spectrophotometer (Model: AA 1275/VARIAN, Australia). The translocation factor (TF) and bioconcentration factor (BF) were calculated as under:

$$\mathrm{TF} = \frac{\text{Cr concentration in shoots}}{\text{Cr concentration in roots}}$$

$$\mathrm{BF} = \frac{\text{Cr concentration in above ground plant parts}}{\text{Cr concentration in soil}}$$

Statistical Analysis

The data were analyzed by performing analysis of variance. The significance of treatments were tested at 0.05 significance level using Duncan's new multiple range test with the help of DSAASTAT software (Onofri Italy).

Results

Soil Analysis

The physicochemical properties of the soil used during this study are described in Table 1.

Cr Tolerance and Growth Promoting Characteristics of *B. fortis* 162

Studies on the basis of maximum threshold limit (MTL) revealed that *B. fortis* 162 was capable to grow up to 300 mg Cr L^{-1}. *B. fortis* 162 was capable to synthesize auxin (64.2 μg 25 mL^{-1} of broth), siderophore (4.8 μg mL^{-1}) and showed positive test for phosphate solubilization.

The ACC is a precursor of ethylene in plants. Reduction in level of stress ethylene due to activity of ACCD help plants to survive heavy metal stress. Our results showed capability of *B. fortis* 162 to hydrolyze ACC by activating ACCD (0.52±0.06 μm α-KB mg^{-1} h^{-1}).

Effect of *B. fortis* 162 on Plant Growth under Cr Stress

During current study, growth response of *C. roseus* plants growing in different concentration of Cr was evaluated in presence/absence of *B. fortis* 162. The reduced plant growth was observed at correspondingly higher concentrations of Cr in case of both un-inoculated plants and those co-cultivated with *B. fortis* 162 (Table 2).

Maximum root length (8.8 cm) was observed in inoculated plants grown in C0. Un-inoculated plants grown in C1, C2 and C3 showed 33, 93 and 130% lower root length respectively, as compared to un-inoculated control plants. Whereas, inoculated plants grown in C0, C1, C2 and C3 showed 6, 17, 26, and 36% higher root length respectively, as compared to un-inoculated plants growing in analogous Cr concentrations. The maximum shoot length (29 cm) was observed in inoculated plants growing in C0, which was significantly higher as compared to all other treatments. Similarly, inoculated plants produced significantly higher shoot length (Table 2) as compared to corresponding un-inoculated *C. roseus* plants.

Quantification of Proline and Chlorophyll Contents

The effect of different Cr concentrations on proline and chlorophyll contents is shown in Fig. 1. Application of *B. fortis* 162 improved growth and chlorophyll contents in *C. roseus* plants growing in either concentration of Cr. The inoculated and un-inoculated plants exhibited lower chlorophyll contents at higher concentrations of Cr as compared to their respective control. The inoculated plants in case of C0, C1, C3 and C3 produced 12, 20, 34, and 27% higher chlorophyll contents as compared to un-inoculated plants subjected to similar Cr concentration.

Table 1: Physicochemical properties of soil

Soil property	value
Total potassium	2.58 g kg^{-1}
Total phosphorus (P)	0.74 g kg^{-1}
Total cadmium (Cd)	0.39 mg kg^{-1}
Total zinc (Zn)	45.16 g kg^{-1}
Total nitrogen (N)	1.47 g kg^{-1}
Total chromium (Cr)	2.7 mg kg^{-1}
Total organic matter	3.2 g kg^{-1}
pH	7.5

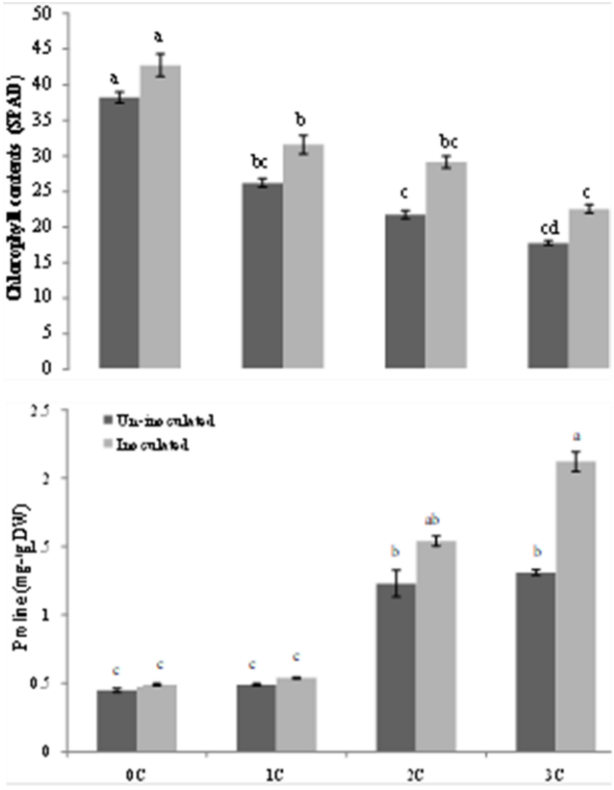

Fig. 1: Effect of *B. fortis* 162 on proline and chlorophyll contents of *C. roseus* grown in Cr-contaminated soil. Data is mean± SD (*n*=3), Treatment means with common letters do not differ significantly at $p < 0.05$, C0= Soil without Cr amendment, C1= 50 mg Cr kg^{-1} soil amendment, C2= 100 mg Cr kg^{-1} soil amendment, C3= 200 mg Cr kg^{-1} soil amendment, DW= Dry weight

The induced stress resistance increased proline content in plants. In our research, higher proline contents were observed in case of higher concentrations of Cr. Moreover, proline contents in plants growing in similar Cr concentration were higher in case of *B. fortis* 162 inoculated plants as compared to un-inoculated ones (Fig. 1). The maximum proline contents (2.2 mg/g DW) were recorded in inoculated plants subjected to C3, which was significantly higher to rest of the treatments. The inoculated plants grown in C0, C1, C2 and C3 exhibited 8, 10, 25 and 61% higher proline contents as compared to un-inoculated plants.

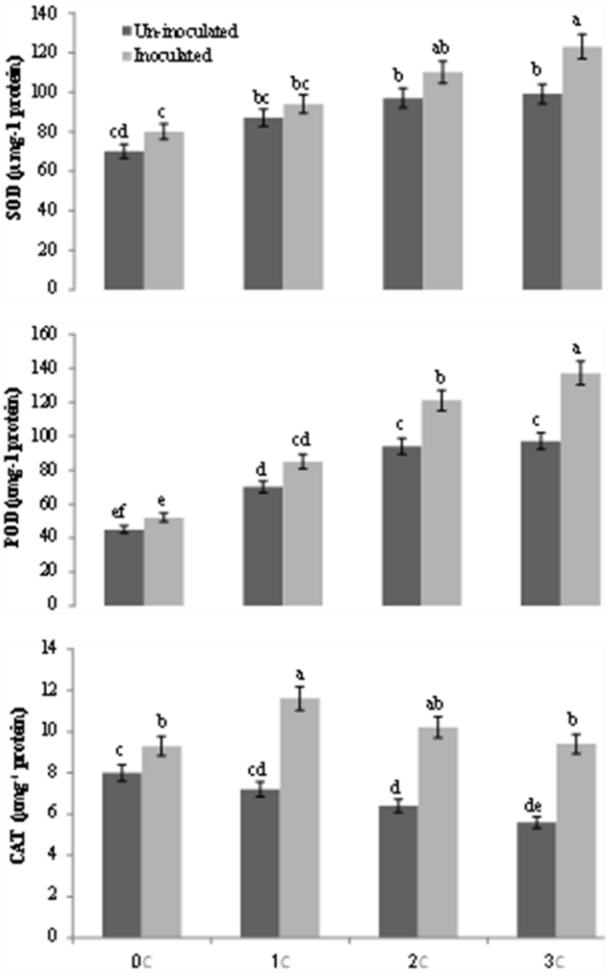

Fig. 2: Effect of *B. fortis* 162 on superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) contents of *C. roseus* grown in Cr-contaminated soil. Data is mean± SD (*n*=3), Treatment means with common letters do not differ significantly at $p< 0.05$, C0= Soil without Cr amendment, C1= 50 mg Cr kg^{-1} soil amendment, C2= 100 mg Cr kg^{-1} soil amendment, C3= 200 mg Cr kg^{-1} soil amendment

Assay of Enzyme Activities

It was observed that inoculated *C. roseus* plants exhibited significantly improved activity of antioxidants enzymes including SOD, POD and CAT. On the other hand un-inoculated plants under Cr stress showed reduced activity of CAT and improved activity of SOD and POD as compared to un-inoculated control. The increased SOD activity in case of un-inoculated C1, C2 and C3 plants was 28, 42 and 50% respectively as compared to un-inoculated control plants. The *B. fortis*162 inoculation under Cr stress (C1, C2 and C3) enhanced 5, 15 and 28% SOD activity as compared to corresponding un-inoculated plants (Fig. 2).

Table 2: Effect of *B. fortis* 162 on root length, shoot length and dry biomass of *C. roseus* grow in Cr-contaminated soil

Treatments	Root length (cm)		Shoot length (cm)		Dry weight (g)	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated	Un-inoculated	Inoculated
C0	8.3±0.3ab	8.8±0.65a	25±1.3ab	29±1.5a	1.3±0.05ab	1.48±0.08a
C1	6.2±0.5bc	7.3±0.57b	16±2c	23±1.8b	0.9±0.07cd	1.25±0.05b
C2	4.3±0.32cd	5.4±0.43c	14±2.3cd	19±2.3bc	0.7±0.06e	0.95±0.04c
C3	3.6±0.25d	4.9±0.25c	11±1.5d	16±1.5c	0.55±0.03f	0.81±0.03d

Data is mean± SD (n=3), Treatment means with common letters do not differ significantly at $p< 0.05$, C0= Soil without Cr amendment, C1= 50 mg Cr kg^{-1} soil amendment, C2= 100 mg Cr kg^{-1} soil amendment, C3= 200 mg Cr kg^{-1} soil amendment

Table 3: Effect of *B. fortis* 162 on the level of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents in *C. roseus* grown in Cr-contaminated soil

Treatments	MDA (nmol g^{-1} FW)		H_2O_2 (ng g^{-1} FW)	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated
C0	7.5±1.3d	6.5±1.5de	75±3.5f	84±2.0e
C1	11±1.5c	9±1.8cd	102±2.4c	94±2.95d
C2	14±1.7cb	12±2.3bc	123±3.6ab	105±3.1bc
C3	18±1.5a	14±1.5b	135±4.0a	115±3.45b

Data is mean± SD (n=3), Treatment means with common letters do not differ significantly at $p< 0.05$, C0= Soil without Cr amendment, C1= 50 mg Cr kg^{-1} soil amendment, C2= 100 mg Cr kg^{-1} soil amendment, C3= 200 mg Cr kg^{-1} soil amendment, FW= Fresh weight

The POD activity in un-inoculated plants was 40, 96 and 90% higher in C1, C2 and C3 as compared to un-inoculated control. Similarly, POD activity in inoculated C0, C1, C2 and C3 plants increased 10, 17, 8 and 31% respectively (Fig. 2) as compared to un-inoculated ones. Reduced CAT activity (28, 50 and 80%) was observed in un-inoculated C1, C2 and C3 plants respectively as compared to un-inoculated control plants. The bacterial inoculation improved 12, 71, 66 and 80% CAT activity in C0, C1, C2 and C3 plants respectively as compared to un-inoculated ones (Fig. 2).

Changes in Level of MDA and H_2O_2 Contents

Variations in MDA content help to determine lipid peroxidation level of membranes exposed to ROS. Oxidative damage due to Cr stress was determined by evaluating the variations in indigenous quantity of H_2O_2 and MDA. Generally, a significant change in MDA contents of plants growing in Cr contaminated soil in presence and absence of *B. fortis* 162 was observed as compared to the plants growing in non-contaminated soil. The MDA contents in un-inoculated C1, C2 and C3 plants increased 46, 86 and 140%, while *B. fortis* 162 inoculation (C1, C2 and C3) demonstrated reduced membrane damage with 20, 60 and 86% decrease respectively in MDA contents as compared to un-inoculated control (Table 3). The Cr induced changes in H_2O_2 contents of inoculated and un-inoculated plants are presented in Table 3. Significantly reduced level of H_2O_2 contents was observed in *B. fortis* 162 inoculated C1 and C3 plants as compared to those inoculated with bacteria in absentia of Cr. The un-inoculated C3 plants exhibited 80% higher H_2O_2 contents, while inoculated C3 plants showed 53% higher level of H_2O_2 contents as compared to un-inoculated control (Table 3).

Assessment of Plant Biomass

To evaluate the effect of *B. fortis* 162 inoculation on *C. roseus* biomass, dry weight of *C. roseus* was calculated in plants under Cr stress. The inoculated plants showed higher plant biomass in all concentrations of Cr as compared to un-inoculated ones (Table 2). A linear decrease in dry weight of *C. roseus* was observed with subsequent increased concentration of Cr in all cases. Similarly, un-inoculated plants showed lower quantity of dry weight as compared to inoculated ones. Least dry weight was observed in un-inoculated plants grown in 200 mg Cr kg^{-1} soil, whereas, highest quantity of dry weight was exhibited by inoculated plants treated with C0. The inoculated plants in case of C0, C1, C2 and C3 produced 10, 40, 25 and 56% higher dry weight respectively, as compared to un-inoculated plants (Table 2). These results indicate that Cr stress may decrease growth and biomass in *C. roseus* plants. Moreover, these results depict that *B. fortis* 162 acts as growth promoter and help in alleviation of Cr stress.

Cr uptake in *C. roseus* Plants

The present study demonstrated that *B. fortis* 162 has a key role in Cr uptake in *C. roseus* plants grown under Cr stress (Fig. 3). Subsequent higher Cr uptake was observed in un-inoculated and inoculated *C. roseus* plants at higher concentration of Cr. The inoculated *C. roseus* plants subjected to C3 showed maximum Cr contents which were significantly higher to all other treatments. The *C. roseus* plant exhibited more Cr accumulation in roots as compared to shoots. The higher Cr contents were observed in *B. fortis* 162 inoculated plants as compared to their corresponding un-inoculated ones. The *B. fortis* 162 inoculated plants exhibited 29, 27 and 32% more Cr content in shoots in case of C1, C2 and C3, respectively as compared to un-inoculated plants treated accordingly. The enhanced values for TF and BF were recorded in inoculated plants as compared to un-inoculated ones (Table 4).

Table 4: Effect of *B. fortis* 162 on Translocation Factor (TF) and Bioconcentration Factor (BF) in *C. roseus* grown in Cr-contaminated soil

Treatments	TF		BF	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated
C0	0.01±0.001e	0.01±0.001e	0.01±0.001d	0.01±0.001d
C1	0.65±0.03c	0.72±0.06b	0.31±0.04bc	0.42±0.05a
C2	0.73±0.05ab	0.79±0.07a	0.28±0.03c	0.39±0.04ab
C3	0.57±0.04d	0.72±0.05b	0.23±0.02cd	0.35±0.03b

Data is mean± SD (n=3), Treatment means with common letters do not differ significantly at $p< 0.05$, C0= Soil without Cr amendment, C1= 50 mg Cr kg^{-1} soil amendment, C2= 100 mg Cr kg^{-1} soil amendment, C3= 200 mg Cr kg^{-1} soil amendment, FW= Fresh weight

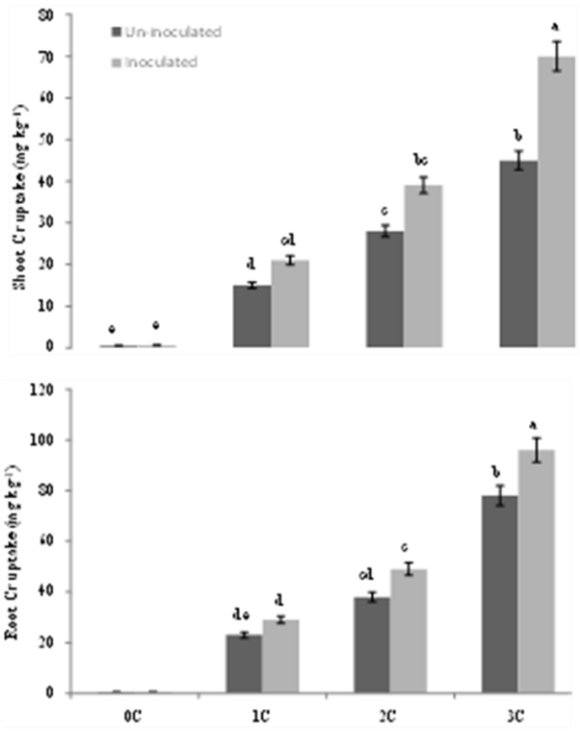

Fig. 3: Effect of *B. fortis* 162 on Cr uptake in *C. roseus* grown in Cr-contaminated soil. Data is mean± SD (n=3), Treatment means with common letters do not differ significantly at $p< 0.05$, C0= Soil without Cr amendment, C1= 50 mg Cr kg^{-1} soil amendment, C2= 100 mg Cr kg^{-1} soil amendment, C3= 200 mg Cr kg^{-1} soil amendment

Discussion

At present bioremediation is believed to be an effective technique for restoration of contaminated area. The present results indicate that metal toxicity impede metabolic activities of inoculated and un-inoculated plants resulting in decreased biomass production (Sobariu *et al*., 2016). Bacteria induce plant resistance against heavy metals and help in growth promotion (Dourado *et al.*, 2013). The combination of plants and metal resistant PGPR may be used effectively for remediation of Cr polluted soils (Rajkumar *et al.*, 2012). The ACCD synthesized by PGPR decreases ethylene production and improves growth of treated plants by consuming ACC which is a precursor of ethylene (Glick *et al.*, 2007). The ACCD hydrolyses ACC into ammonia and ketobutyrate resulting in induced systemic tolerance in plants and subsequently increased plant growth (Honma and Shimomura, 1978; Glick, 2014). The microbes having ACCD activity enhance nutrients uptake in plants and have a significant role in growth of plants growing under environmental stress (Nascimento *et al*., 2014). Bacteria with ACCD activity improve metal accumulation in plant parts and result in improved root and shoot growth (Belimov *et al*., 2005).

During our present study, the effect of *B. fortis* 162 inoculated *C. roseus* plants at various levels of Cr was observed to evaluate the potential of plant-microbe interaction for bioremediation of Cr affected soils. Generally, the growth parameters for example root length, shoot length and dry biomass are regarded as markers of plant growth under stress conditions (Chen *et al*., 2008). During current research, gradual decrease in root and shoot growth of un-inoculated *C. roseus* resulted at subsequent higher concentration of Cr. On the other hand, enhanced shoot and root length was found in bacteria inoculated *C. roseus* plants. Whereas, plants growing in lower Cr concentration showed improved growth (Table 2). The metal toxicity in contaminated soils may reduce plant growth (Yang *et al*., 2010; Singh *et al*., 2016). However, growth promoting bacteria such as *B. fortis* 162 may help plants to resolve the issue. Rajkumar and Freitas (2008), found that rhizospheric bacteria inoculated plants growing under heavy metal contaminated soils exhibited over 30% shoot and root length as compared to un-inoculated ones.

The PGPR help associated plants to overcome environmental stress and improve root and shoot growth (Egamberdiyeva and Höflich, 2004). The beneficial effects of these microbes on inoculated plants may be credited to the activity of ACCD which is capable to hydrolyze ACC and synthesis of auxin, which increase growth parameters under Cr stress. The Cr stress exhibited a detrimental effect on shoot length, root length and biomass of *C. roseus* plant as compared to plants growing under non contaminated soil. The current study demonstrated that *B. fortis* 162 decreased injurious effects of Cr and consequently increased Cr accumulation and plant growth in inoculated *C. roseus* plants as compared to un-inoculated plants. Rocca *et al*. (2009), found that heavy metal stress impede physiological activities of bacteria inoculated and un-inoculated plants resulting in decreased biomass production of these plants. The quantity of reactive oxygen species increases in plants growing under metal stress. These ROS have negative effect on metabolic activities of plants. Rajkumar and Freitas (2008) found that different PGPR may synthesize growth promoting hormones and biochemicals. These hormones such as auxins and gibberellins improve plant growth, development and biomass under heavy

metal stress (Dell'Amico *et al*., 2008; Baharlouei *et al*., 2011). The bacterial hormones initiate plants to synthesize phytohormones capable to mitigate toxicity of heavy metal concerned.

Leaf chlorophyll help plants to prepare their food during the process of photosynthesis. Heavy metals may inhibit biosynthesis of chlorophyll due to hindrance in photosynthetic electron transport chain (Patsikka *et al*., 2002). In our current study *B. fortis* 162 inoculated plants exhibited more chlorophyll contents as compared to un-inoculated plants growing under Cr stress. Therefore, our results are in accordance with the observations of Zhang *et al*. (2011), indicating increased chlorophyll contents in PGPR inoculated plants growing in heavy metal contaminated soils. Iron (Fe) is an important constituent of chloroplast, heavy metals stress reduce Fe uptake in plants (Ma and Nomoto, 1993). Therefore, Fe deficiency decreases photosynthetic activities in affected plants (Imsande, 1998). Siderophore producing bacteria assist plants to acquire Fe from rhizospheric area (Burd *et al*., 2000). The improved Fe uptake enhances leaf chlorophyll and photosynthetic activity of plants.

During current study, significantly enhanced proline contents were observed in case of inoculated plants grown at higher concentrations of Cr. Proline is an important biomarker of plant resistance against environmental stress. Our results confirm the role of *B. fortis* 162 to survive in Cr contaminated soil and enhanced proline contents verify improved resistance level of *C. roseus* against Cr stress. Glick *et al*. (1998) also observed higher proline contents in heavy metal tolerant plant species as compared to susceptible ones. Handique and Handique (2009) also found increased proline contents in *Cymbopogon flexuosus* plants grown under heavy metal stress. John *et al*. (2009) reported that higher quantity of proline is synthesized in microorganism and plants under abiotic stress. The proline helps organisms to escape abiotic stress by decreasing quantity of ROS, stabilization of stress enzymes and buffer cellular redox (Ahmad *et al*., 2006). Mattioni *et al*. (1997) reported that higher quantity of proline under stress may be attributed to its reduced breakdown or improved biosynthesis. Janmohammadi *et al*. (2013) also found increased proline level in PGPR associated *Triticum aestivum* plants subjected to heavy metal stress. During our current trial, enhanced proline level was noted in bacteria inoculated *C. roseus* plants treated with different concentrations of Cr. Similarly, increased proline level was observed with constant increase in Cr concentration in case of inoculated and un-inoculated plants. The increased level of proline in *C. roseus* plants during current study may be attributed to increased stress as per findings of Ahmad and Jhon (2005). During stress, proline helps in regularization of cellular chemistry and osmotic potential in plant tissues.

The increased toxic effects on *C. roseus* plant at higher Cr concentration confer that metal excess damages tissues by causing oxidative damage through the generation of ROS (Noctor *et al.*, 2016). The antioxidant enzymes activities in different plant species grown in contaminated soil depend on concentration, exposure duration and kind of metal ions. These enzymatic changes reflect the modified redox status of the stressed tissues (Sharma and Dietz, 2008). The SOD converts superoxide radicals in stressed plants to H_2O_2 and plays a significant role in the protection of the cells against the injurious effect of ROS (Verma and Dubey, 2003). Improved SOD activity enhance the concentration of superoxide radical that is likely due to the formation of enzymatic proteins (Verma and Dubey, 2003; Kumar and Verma, 2018). The inoculated plants growing in contaminated soil exhibited potential of *B. fortis* 162 to generate higher quantity of SOD (Islam *et al.*, 2014).

POD helps in the detoxification of H_2O_2. In present study, higher POD activity was also observed in inoculated plants as compared to un-inoculated plants growing in similar concentration of Cr (Erdogan *et al.*, 2016). CAT is involved in the conversion of H_2O_2 into water. Various plants exhibit increased or decreased activity of CAT under the influence of metal stress (Zhao and Zhang, 2006). During current research decrease in CAT activity was observed in *C. roseus* plants under Cr stress. Taran *et al.* (2016) have also reported the reduced level of CAT activity in plants subjected to heavy metal stress. In our study, improved CAT activity in inoculated *C. roseus* plants was observed under Cr stress. Wang *et al.* (2011) also recorded enhanced level of CAT activity in *Populus deltoides* LH05-17 by inoculation of *Agrobacterium radiobacter* under Arsenic (As) stress. It was observed that SOD and CAT activity increased significantly in *B. fortis* 162 inoculated plants under Cr stress which indicates induction of oxidative stress tolerance through ascorbate-glutathione pathway (Shereefa and Kumaraswamy, 2016). PGPR enhance gene/mRNA expression of plant antioxidant enzymes resulting in improved activity of antioxidant enzymes, despite decrease in MDA and H_2O_2 in inoculated plant as compare to un-inoculated plants growing under metal stress (Gururani *et al.*, 2012).

Most of the metals on earth crust usually exist in their insoluble form (Lasat, 2002). PGPR help in bioavailability of these metals due to which plants may uptake higher quantity of these metals (Abou-Shanab *et al*., 2006). The enhanced metal uptake and reduced metal toxicity in inoculated plants is due to siderophore production, phosphate solubilization and synthesis of chelating biochemicals (Tak *et al.*, 2013). The decreased Cr uptake in un-inoculated plants during current research may be due to phytotoxicity of Cr in *C. roseus* plants. *B. fortis* 162 may reduce Cr toxicity which may be correlated to decreased concentration of stress ethylene. It is a well-known fact that reduced level of stress ethylene lowers stress induced injuries in plants exposed to environmental stress (Van-Loon *et al.*, 2006). However, growth promoting attributes of *B. fortis* 162 decreased Cr toxicity resulting in

increased accumulation of Cr in root and shoot tissues. Our results are in accordance with the observations of Yousaf *et al*. (2010). The improved TF and BF in inoculated plants may be attributed to enhanced metal bioavailability and reduced metal toxicity due to growth promoting traits of rhizobacteria (Mohammadzadeha *et al.,* 2016). PGPR may produce redox change; chelates, organic acids and convert non soluble form of metal into soluble form resulting in enhanced metal uptake in shoot and root tissues of the plants. Therefore, growth promoting attributes of *B. fortis* 162 may have enabled enhanced growth, stress tolerance and Cr uptake in inoculated *C. roseus* plants. Our results suggest that *B. fortis* 162 may be inoculated in plants to enhance their growth and phytoextraction potential in Cr polluted soils.

Conclusions

Present study revealed that *B. fortis* 162 inoculation enhanced growth, oxidative stress tolerance and Cr uptake in *C. roseus* cultivated in Cr contaminated soil. These results explore better opportunity for understanding plant-microbe interaction under Cr stress.

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