Journal of Plant Biology, June 2005, 48(2) : 187-194

Chromate Resistant *Bacillus cereus* Augments Sunflower Growth by Reducing Toxicity of Cr (VI)

Muhammad Faisal and Shahida Hasnain*
Department of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan

Impact of four chromium resistant bacterial strains (S3, S4, S6, and S7) was studied on the different growth parameters of sunflower (*Helianthus annuus* var SF-187) in chromium free or under chromium stress. Strains used exhibited very high-level resistance to chromate (up to 50 mg ml^{-1} on nutrient agar and 1-2 mg ml^{-1} in minimal medium). Application of Cr(VI) salt adversely affected the seed germination, root and shoot length, and fresh weight of seedlings. Bacterial inoculations improved the growth parameters. The effects of Cr(VI) on the different biochemical parameters were also very severe but seedlings inoculated with bacteria showed much improvements as compared to non-inoculated controls. Uptake of Cr(VI) was higher than Cr(III) by the seedlings. Inoculated seedlings contained less chromium than non-inoculated seedlings. Much improvement in the internal region of root and shoot was observed in inoculated plants especially in guard cells.

Keywords: Bacillus cereus, chromium, heavy metals, plant anatomy, plant growth promotion, sunflower

Soil has been contaminated with several heavy metals mostly from mining wastes and industrial discharges. Heavy metals are often toxic in both the chemically combined and the elemental form. Several approaches have been taken to clean up contaminants that had been relying on microorganisms, plants, and chemical treatments. Plant based bioremediation depends on the ability of plant to survive in the presence of contaminants, to metabolize or exclude contaminants, and to provide nutrient supplements to microbes that can accelerate degradation of contaminant.

One common polluting metal is chromium, arising from discharged effluents from leather tanning, electroplating, and alloy preparation (Stoychev et al., 2002). Because of its widespread use and its adverse impact on the environment, much attention has been on chromium from national and international organizations. Even though chromium is an essential component for normal glucose utilization, chromium at high concentration becomes problematic for both fauna and flora (Proctor et al., 2002). Chromium commonly exists as Cr(III) and Cr(VI), and Cr(VI) is more mobile and permeable. It can be easily taken up by plants and subsequently can enter into the food chain. Several bacterial strains have been identified that can reduce Cr(VI) to Cr(III), a form less mobile and permeable (Mabbett et al., 2002). To confirm the possibility that chromium resistant bacteria might also contribute in plant growth by chelating or reducing the toxic Cr(VI) to less permeable Cr(III) form, the present work was carried out. Here we report the effect of four chromium resistant bacterial strains on the germination and growth of sunflower under Cr(III) and Cr(VI) stresses.

*Corresponding author; fax +92-42-9230481
e-mail genetic@brain.net.pk

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Four chromium resistant bacterial strains S3, S4, S6 and S7 were isolated from chromium-contaminated soil. Strains S-3, S-4 and S-6, S-7 used in this study were isolated from chromium contaminated soil of Kasur and Muridkey, Lahore, Pakistan, respectively. These strains were isolated and purified at 1000 μg ml^{-1} of potassium chromate supplemented medium. Strains were stored at 4°C.

Germination Experiments

Sunflower (*Helianthus annuus* var SF-187) seeds were collected from the Monsanto sub-office in Lahore, Pakistan. Seeds were surface-sterilized in 5% sodium hypochlorite for 5 min and then thoroughly washed with sterilized glass-distilled water thrice. Two different chromate salts were used (trivalent chro-

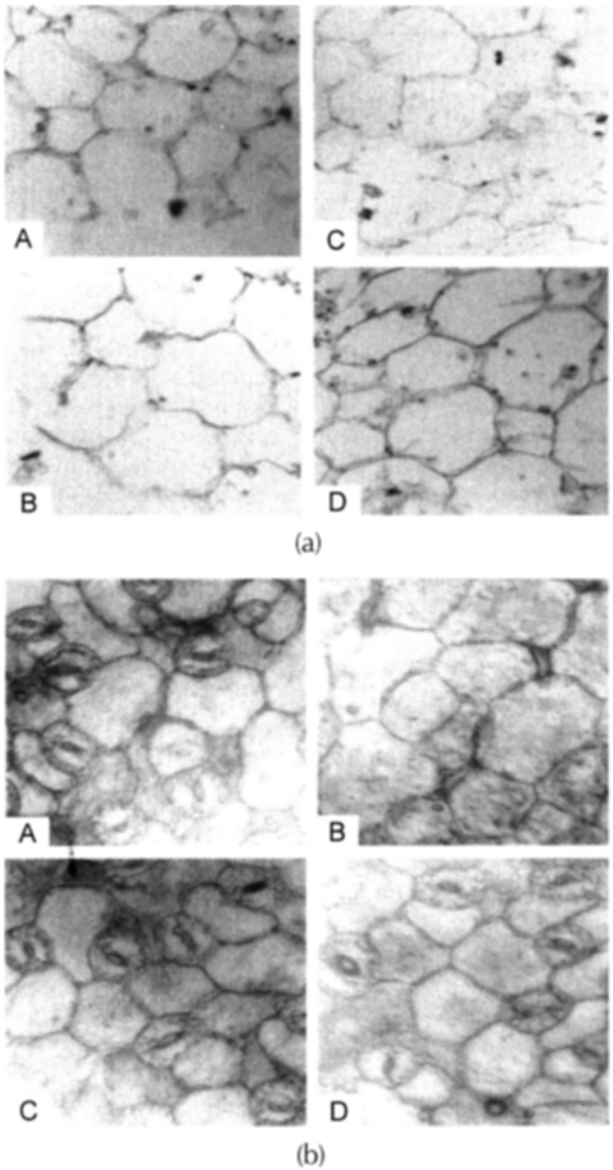

Figure 1. a) Effect of bacterial strains and chromate salt on the pith region of stem of *H. annuus* var. SF-187 plants. **A**, control at 0 μg ml^{-1} K_2CrO_4; **B**, innoculated at 0 μg ml^{-1} K_2CrO_4; **C**, control at 300 μg ml^{-1} K_2CrO_4; **D**, inoculated at 300 μg ml^{-1} K_2CrO_4. bar = 10 μm. b) Effect of bacterial strains and chromate salt on stomatal morphology of seedlings of *H. annuus* var. SF-187. **A**, control at 0 μg ml^{-1} K_2CrO_4; **B**, inoculated at 0 μg ml^{-1} K_2CrO_4; **C**, control at 300 μg ml^{-1} K_2CrO_4; **D**, inoculated at 300 μg ml^{-1} K_2CrO_4. bar = 10 μm.

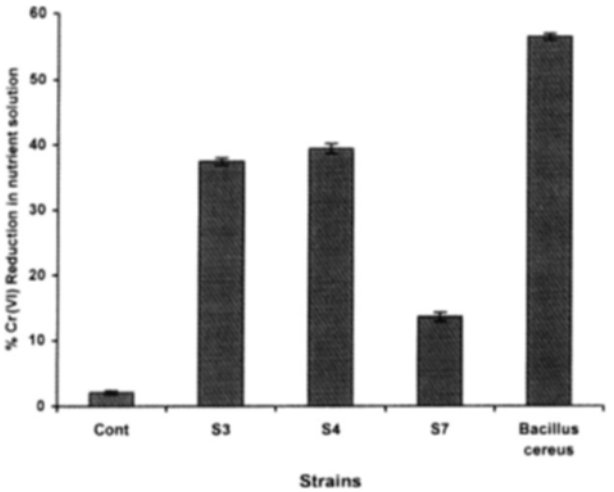

Figure 2. Cr(VI) reduction by the bacterial strains and sunflower seedlings present in the nutrient solution within 10 days. In controls, only sunflower seedlings present, and in other four plates, plants were inoculated with the bacterial strains.

mium $CrCl_3$ and hexavalent K_2CrO_4, 300 μg ml^{-1}). For seed inoculation, freshly prepared overnight bacterial cultures were suspended in 10 ml sterilized glass distilled water, and the titers were adjusted to A_{600} = 1.2. Both inoculated and un-inoculated (control) seeds (20 seeds per plate) were spread uniformly on the filter papers in Petri dishes (Afrasayab et al., 2001). Seeds were germinated in the dark. After germination, seedlings were nourished with nutrient solution (Hewitt, 1963) supplemented with respective chromate salts ($CrCl_3$ or K_2CrO_4 at 300 μg ml^{-1} each), and cultured in the light with 12 h photoperiod using tube light. After 10 days, seedlings were harvested, and seed germination rate, root and shoot length, number of roots, fresh weight and dry weight of seedlings were measured. Activity of acid phosphatase was measured following Iqbal and Rafique (1986). For the determination of chromium content in sunflower, seedlings were thoroughly rinsed with distilled water, placed in between filter papers to remove excess water and dried at 80°C for 24 h in an oven (Wisconsin Oven Distributors, Germany). Dried material was crushed to small pieces, and chromium in the tissue was analyzed by the acid digestion method (Humphries, 1975). Chromium content was determined following Clesceri et al. (1998). Each treatment was repeated four times.

Pot Experiments

Experiments were also conducted in pots, each containing 3 kg of garden soil. To observe the effect of different chromate salt, pots were also amended with $CrCl_3$ or K_2CrO_4 (300 μg g^{-1}). Seeds were inoculated as described above. Ten seeds were sown in each pot and watered after every 24 h. Ten days after germination, plants were

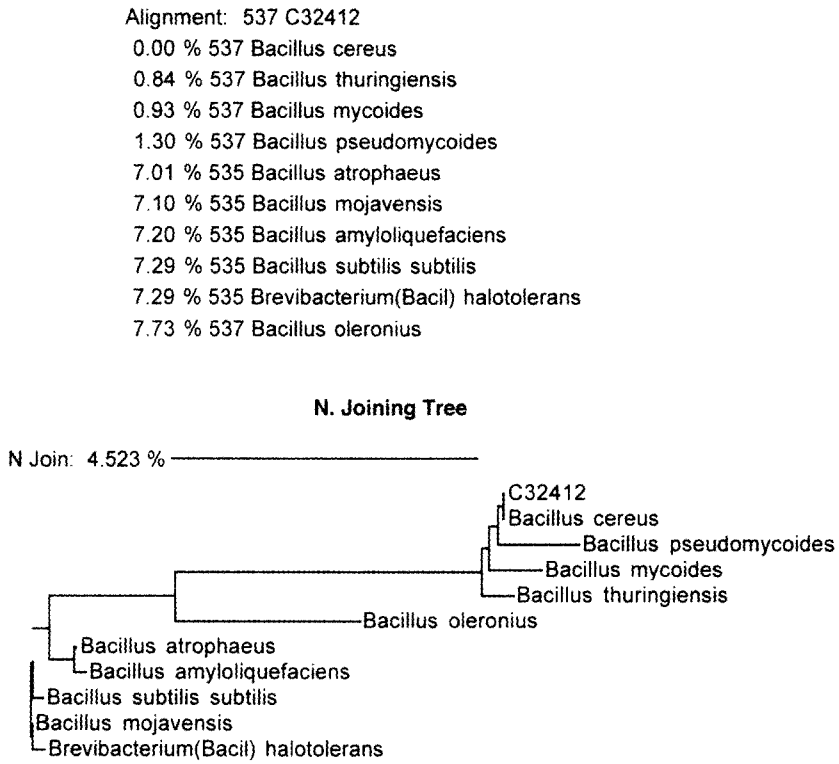

Figure 3. Phylogenetic dendrogram of strain S6 (C32412) that was identified as *B. cereus* S-6.

thinned to four plants per pot. Plants were harvested at full maturity. Chromium content in the plants grown in pots was also determined as described above.

Anatomical Study

One cm samples of root and shoot were embedded in paraffin wax and transversely sectioned in 10-12 μm thick. Any effect posed by the bacteria and chromium salts on the internal anatomy of seedlings of sunflower was observed under a compound microscope (Sanderson, 1994). For root, diameter of cortex, vascular bundle, epidermis and for shoot, diameter of cortex, vascular bundle and pith were studied.

Reduction of Chromate

Seedlings, control and the inoculated, were grown in the presence of 300 μg ml^{-1} of K_2CrO_4. After ten days, seedlings were harvested and the amount of chromate reduced was determined in the nutrient solution.

Sequencing of 16S rRNA Gene

To confirm taxonomic identity of the strain S6, which showed better sunflower growth promotion, 16S rRNA gene was sequenced. To this end, DNA was extracted. Chromium resistant bacterial strains were grown in BHI broth (Difco Laboratories, USA) and DNA extracted by using The Genomic DNA Isolation Kit (Fermantas, USA) according to manufacturer's instructions. DNA concentration was measured spectrophotometrically. The 16S rRNA genes (rDNA) were amplified by using universal primers p27f and p1525r. Each PCR mixture (100 ml) contained primers (each at a concentration of 0.4 mM), a mixture of deoxynucleoside triphosphates (Fermantas) (each at a concentration of 200 mM), and *Taq* polymerase buffer (Fermantas). Chromosomal DNA (ca. 100 ng) was added to the solution, which was then heated at 94°C for 2 min and cooled immediately on ice. *Taq* polymerase (2.5 U) and 1 drop of mineral oil (Sigma, USA) was then added to each of the reaction solutions. The DNA thermal cycler (Master Cycler, UK) used for thermal amplification was programmed as follows: (i) an initial extensive denaturation step consisting of 94°C for 2 min; (ii) 25 reaction cycles, with each cycle consisting of 94°C for 1 min, 55°C for 1 min, and 72°C for 3 min; and (iii) a final extension step consisting of 72°C for 10 min. The extension product was then separated on an ABI PRISM® (automated DNA sequencer) and the data was com-

pared to the MicroSeq® databases (ACCUGENIX™ Newark DE 19702).

RESULTS

Four chromium resistant bacterial strains (S3, S4, S6 and S7) were isolated from the soil highly contaminated with chromium. All bacterial strains could resist up to 50 mg ml^{-1} of K_2CrO_4 on nutrient agar medium and 1-2 mg ml^{-1} on M9 minimal medium. Strains S3 and S4 were gram negative rods while strains S7 and S6 were gram positive cocci and rod, respectively. Strains S6 and S7 shared most of their characteristics with the genus *Staphylococcus* and *Bacillus*, respectively. Identification of isolate S6 based on its partial gene sequence (500 bp 16S rRNA) placed it as *Bacillus cereus* S-6 (Fig. 3).

Application of both chromate salts caused significant reduction in seed germination especially in case of Cr(VI) when compared with the control (Table 1). Bacterial inoculation resulted in an enhancement in seed germination both under $CrCl_3$ and K_2CrO_4 treatments. Both chromate salts adversely affected root and shoot lengths (Table 1). Significant decrease in the root length (73.61%) was observed at 300 μg ml^{-1} K_2CrO_4. Cr(III) was less toxic and only 6.13% decrease in root length was observed. Interestingly, all the bacterial strains promoted root length even in the presence of toxic Cr(VI) salt. When there was no chromium, maximum enhancement was observed with the strain S6 that improved the root length by 24.53%. In the presence of Cr(III) and Cr(VI), improvement in the root length by S6 strain was 26.14% and 46.51%, respectively. Strain S6 also significantly improved shoot length (Table 1). All strains except S7 improved shoot length in the presence as well as in the absence of chromate. Maximum

Table 1. Effect of chromium-resistant bacteria on germination and root and shoot length. Seedlings of *H. annuus* var. SF-187 at 0 or 300 μg ml^{-1} of $CrCl_3$ or K_2CrO_4 were inoculated. Means of three replicates are presented.

STRAINS	% Germination			Root Length (cm)			Shoot Length (cm)		
	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4
Control	100±0.00	92±2.48	80±3.49	1.63±0.08	1.53±0.05	0.43±0.06	7.46±0.82	7.20±0.68	2.60±0.06
S3	100±0.00	100±0.00	85±3.75	1.76±0.06	1.60±0.09	0.50±0.05	9.80±0.85	7.73±0.60	2.73±0.07
S4	100±0.00	95±2.12	82±3.94	1.80±0.05	1.70±0.06	0.53±0.06	8.70±0.48	8.06±0.90	2.93±0.09
S6	100±0.00	100±0.00	90±3.18	2.03±0.08	1.93±0.06	0.63±0.06	9.53±0.80	8.60±0.72	3.53±0.10
S7	100±0.00	89±3.56	80±3.52	1.43±0.09	1.40±0.10	0.30±0.04	6.70±0.46	6.10±0.85	2.10±0.06
LSD at 0.05									
For strain		4.50			0.09			0.92	
For treatment		6.35			0.12			1.19	

Petri plate experiments

Table 2. Effect of chromium-resistant bacteria on fresh weight, dry weight, and acid phosphatase activity. Seedlings of *H. annuus* var. SF-187 at 0 or 300 μg ml^{-1} of $CrCl_3$ or K_2CrO_4 were inoculated with bacteria. Means of four replicates are presented.

STRAINS	Fresh weight (g)			Dry weight/g fresh weight			Acid phosphatases (units/g)		
	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4
Control	4.46±0.34	4.16±0.24	2.11±0.13	0.120±0.01	0.185±0.008	0.242±0.01	31.6±1.50	69.2±2.80	94.5±3.11
S3	5.06±0.30	4.29±0.34	2.13±0.14	0.119±0.008	0.159±0.009	0.193±0.007	29.4±1.54	52.4±2.47	86.3±3.28
S4	4.96±0.51	4.19±0.28	2.19±0.10	0.111±0.009	0.164±0.01	0.203±0.01	32.0±1.80	53.2±2.14	74.0±2.48
S6	5.14±0.42	4.70±0.34	2.49±0.28	0.118±0.007	0.172±0.008	0.148±0.007	32.4±1.21	48.1±1.53	72.8±3.00
S7	4.53±0.35	4.07±0.54	2.14±0.15	0.123±0.02	0.168±0.008	0.230±0.008	27.5±1.60	64.8±1.67	82.1±2.80
LSD at 0.05									
For strain		0.24			0.00			10.9	
For treatment		0.32			0.00			14.1	

Petri plate experiments

decrease in shoot length was 3.48% and 65.14% under 300 μg ml^{-1} of Cr(III) and Cr(VI), respectively.

Cr(VI) had adverse effect on the fresh weight of seedlings. Reduction of 6.72% and 52.69% in fresh weight was observed under trivalent and hexavalent salts, respectively. Strains S3 and S6 enhanced fresh weight up to 13.45% and 68.24%, respectively. Under chromium stress, S6 caused significant increase in the fresh weight (Table 2). Significant increase in dry weight by 54.16% and 101.6% was observed under trivalent and Cr(VI) stresses. In all cases, bacterial strains caused decrease in dry weights. For example, 14.05% decrease in dry weight was observed in S3 inoculated seedlings under Cr(III) stress, while S6 exhibited 38.8% decrease under Cr(VI) stress.

Activity of acid phosphatase increased under the metal stress. Under 300 μg ml^{-1} of $CrCl_3$ and K_2CrO_4, 118.98% and 199.40% increments in acid phosphatase activity were observed. Strains S4 and S6 resulted in an increase, while strains S3 and S7 caused decrease in the activity of acid phosphatase (Table 2).

Under chromium stress, auxin content increased. When there was no chromium stress, all bacterial inoculations caused an increment in auxin content. Maximum increment of 195.2% was observed in the seedlings inoculated with S6 (Table 3). Under Cr(VI) stress, all strains enhanced auxin content, for an example, strain S6 caused 117.2% increment.

Table 3 also showed the uptake of chromate by sunflower seedlings both in the presence of bacterial inoculation and control. Uptake of Cr(VI) was higher

Table 3. Effect of chromium-resistant bacteria on auxin and chromium contents. Seedlings of *H. annuus* var. SF-187 at 0 or 300 μg ml^{-1} of $CrCl_3$ or K_2CrO_4 were inoculated with the bacteria. Means of four replicates were presented.

STRAINS	Auxin contents (μg g^{-1})			Chromium content (mg g^{-1} d.w.)	
	0	$CrCl_3$	K_2CrO_4	$CrCl_3$	K_2CrO_4
Control	0.62±0.12	1.27±0.12	2.15±0.14	0.404±0.04	3.24±0.10
S3	1.21±0.14	1.43±0.14	3.92±0.16	0.402±0.01	2.35±0.20
S4	1.02±0.13	1.28±0.12	4.12±0.10	0.392±0.02	2.37±0.10
S6	1.83±0.15	2.98±0.13	4.67±0.13	0.382±0.03	2.16±0.024
S7	1.19±0.12	1.87±0.10	3.28±0.15	0.393±0.02	2.88±0.02
LSD at 0.05					
For strain		0.77		0.54	
For treatment		1.00		0.70	

Petri plate experiments

Table 4. Effect of chromium-resistant bacteria on root and shoot length, diameter of flower, and chromium content of sunflower plants at 0 or 300 μg g^{-1} of $CrCl_3$ and K_2CrO_4. Means of four replicates are presented.

STRAINS	Root length (cm)			Shoot length (cm)			Diameter of flower (cm)			Chromium content (mg g^{-1} d.w)	
	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4	$CrCl_3$	K_2CrO_4
Control	7.8 ±0.56	6.9 ±0.39	3.4 ±0.4	28.8 ±1.8	26.2 ±1.2	18.9 ±1.27	5.8 ±0.34	5.5 ±0.35	2.1 ±0.11	0.41 ±0.04	2.87 ±0.12
S3	8.6 ±0.43	7.5 ±0.42	3.2 ±0.28	29.4 ±0.97	23.8 ±1.5	19.2 ±0.48	5.9 ±.032	5.4 ±0.32	1.9 ±0.07	0.40 ±0.01	2.76 ±0.014
S4	9.2 ±0.31	7.2 ±0.19	3.9 ±0.18	32.2 ±0.75	28.6 ±0.72	21.4 ±0.38	6.2 ±0.41	5.8 ±0.40	2.2 ±0.12	0.37 ±0.03	2.80 ±0.015
S6	10.3 ±0.91	7.4 ±0.48	4.2 ±0.42	35.9 ±0.86	31.8 ±0.74	24.8 ±0.81	6.4 ±0.29	6.2 ±0.26	2.4 ±0.10	0.38 ±0.01	2.41 ±0.13
S7	8.1 ±0.16	6.5 ±0.27	3.5 ±0.18	29.3 ±1.2	27.7 ±0.89	20.1 ±0.72	5.9 ±0.39	5.6 ±0.24	2.1 ±0.08	0.40 ±0.021	2.0 ±0.10
LSD at 0.05											
For strain		0.83			1.50			0.16		0.45	
For treatment		1.07			1.92			0.21		0.58	

Pot experiments

in all the cases tested compared to Cr(III). The amount of chromate accumulated in the roots was higher than in the shoots and leaves. Bacterial strains significantly reduced uptake of chromate, especially Cr(VI). Strain S6 significantly decreased uptake of Cr(VI) (Table 3).

Results from pot experiments were not as severe as the ones shown from the Petri plates (Table 4). Under K_2CrO_4 stress, root system was severely affected. Bacterial strains significantly enhanced root and shoot lengths. Strain S6 caused an increment of 32.50% in root length. Under 300 μg ml^{-1} K_2CrO_4, 23.52% enhancement in root length was observed in S6 inoculation. Maximum increments of 7.24% and 23.52% in shoot length were observed with strain S6 under Cr(III) and Cr(VI). Consistent increases in all the growth parameters were observed from the inoculated plants with somewhat stronger effects from the strains S4 and S6 compared to the strains S3 and S7. Growth stimulating effects by the bacterial strains were also seen in yield parameters especially in flower diameter. Analysis of chromium content in the sunflower seedlings indicated that bacterial inoculation decreased uptake of chromium. Plants grown under Cr(VI) stress accumulated higher level of chromium than the plants supplemented with Cr(III). Plants inoculated with S4 and S6 strains contained less chromium than the plants inoculated with S3 and S7 strains.

Cr(III) caused some contraction in the diameter of internal regions of stem (Table 5). Stimulatory effects of both chromate salts were more obvious in the cortical and pith regions. However, with bacterial inoculation, diameter of stem showed expansion. Strain S6 increased the diameter of cortical and pith regions by 33.8% and 21.9%, respectively, compared with the respective non-inoculated controls. In case of root, application of both chromate salts resulted in reduction in diameter. Strains S4 and S6 significantly promoted the diameter of epidermal and cortical regions both in chromate-stressed and unstressed conditions. Chromium salts especially Cr(VI) affected the struc-

Table 5. Effect of chromium-resistant bacteria on the internal anatomy of root and shoot of *H. annuus* var. SF-187 plants (15 DAG) at 0 or 300 μg g^{-1} of $CrCl_3$ or K_2CrO_4. Means of four replicates are presented, and variability within means was less than 15%.

a. Root

STRAINS	Diameter of cortex (μm)			Diameter of vascular bundle (μm)			Diameter of epidermis (μm)		
	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4
Control	390	320	350	330	300	290	12.2	12.2	11.28
S3	419	361	372	341	316	297	12.6	12.3	11.56
S4	431	348	383	359	337	299	12.1	12.0	11.28
S6	514	436	439	394	351	328	13.4	12.1	11.68
S7	384	324	362	346	323	289	12.0	14.0	11.24
LSD at 0.05									
For strains		19.3			12.9			1.12	
For Treatment		24.9			16.6			1.45	

b. Shoot

STRAINS	Diameter of cortex (μm)			Diameter of vascular bundle (μm)			Diameter of pith (μm)		
	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4
Control	280	260	230	360	340	360	960	840	890
S3	324	287	261	355	368	371	1040	970	970
S4	316	279	273	372	353	362	1020	880	920
S6	374	367	364	384	384	384	1170	990	1020
S7	301	275	252	381	337	360	1070	860	870
LSD at 0.05									
For strains		19.0			19.8			57.9	
For Treatment		24.6			25.6			74.8	

DAG, days after germination, VB, vascular bundles, pot experiment.

tures (Fig. 1a and b). Cr(VI) resulted in disintegration and deformation of root and shoot cell and tissues while bacterial inoculation not only improved cell diameter but also improved the damaged cells by alleviating the toxic effect of Cr(VI). This releasing effect from the stress was also observed in stomata where Cr(VI) severely damaged the guard cells. Inoculation of bacterial strains under Cr(VI) stress resulted in an improvement in the morphology of guard cells (Fig. 1b).

Figure 2 showed that under control conditions where no bacterial inoculation was applied, sunflower seedlings were basically unable to reduce Cr(VI) to Cr(III). All bacterial strains along with the sunflower seedlings significantly reduced toxic Cr(VI) to less toxic Cr(III) (Fig. 2). Strain S6 was able to reduce Cr(VI) to Cr(III) up to 54.42% while this effect was only 2.06% in non-inoculated control seedlings (Fig. 2).

DISCUSSION

The carcinogenic nature of some heavy metals makes a potential threat to the human health, and chromium is a known human carcinogen (Benova et al., 2002). Some root-associated bacteria and mycorrhizae play an important role in controlling the availability of trace elements for uptake by plant roots (Ehrlich, 1997) in heavy metal contaminated soils. Strains in the present study could grow at very high concentrations of K_2CrO_4. S3, S4 and S7 could resist up to 50 mg ml^{-1} while S6 up to 80 mg ml^{-1} K_2CrO_4 on nutrient agar medium. Such a high level resistance to chromium was not previously reported. These strains not only resisted but also reduced Cr(VI) efficiently. Identification of chromium-resistant bacterial strains could be accessed from the nucleotide sequence of 16S rRNA gene that led this report of Cr(VI) uptake and reduction with a strain of *B. cereus*, i.e., *B. cereus* strain S-6 showed high level resistance to chromate and its subsequent reduction.

Seed germination of *H. annuus* was seriously affected by the presence of Cr(VI) at 300 μg ml^{-1}. Similar findings were also observed by Mehmood et al. (1998) where germination of *Triticum aestivum* seedlings was markedly affected by the presence of chromate salts. Damage by the toxicity was more prominent in the root. Cr(VI) caused decrease in root length and number of root hairs, but in the inoculated plants this effect was less drastic. Application of 300 mg ml^{-1} of Cr(III) did not result in apparent toxic symptoms both in the inoculated and control plants. In all cases, bacterial inoculations significantly enhanced plant fresh weight, root and shoot lengths, and number of root hairs. Reduction in fresh weight of sunflower seedling under Cr(VI) stress might be due to reduced uptake of water. Heavy metal toxicity hampers cell division and decreases turgor pressure of plant cell (Kastori et al., 1992). Fresh weight of *Salvinia minima* was significantly reduced as the Cr(VI) concentration increased in the growth medium (Nichols et al., 2000).

In the sunflower seedling, the activity of acid phosphatase was affected under chromium stress. Acid phosphatase releases phosphate from substrates, and the released phosphate is a major source of inorganic phosphorous in plants (Wyss et al., 1998). Bacterial strains have been reported to stimulate acid phosphatase and may secrete acid phosphatase (Saleh and Belisle, 2000).

Chromium content in the inoculated plants with bacteria was less than those found in the corresponding controls. These effects were more evident in case of Cr(VI) where all bacterial strains decreased the metal availability to the plant by reducing its most soluble and permeable form, i.e., Cr(VI) to a less permeable Cr(III). Hence bacterial strains stimulate growth parameters of plants by minimizing the toxic effects of this metal in contaminated soil.

According to Burd et al. (2000), bacteria can improve plant growth by lowering the level of ethylene in plants, thereby allowing plants to develop larger roots. This will help the plant to acquire more iron for growth in the high level of heavy metals, which might otherwise make the acquisition of iron difficult. Hoflich and Metz (1997) observed that some plant growth promoting bacteria were able to stimulate maize growth in soil polluted with heavy metals containing sewage. Our previous work (Hasnain and Sabri, 1997; Mehmood et al., 1998) also reflected growth improvement of wheat and mung bean by inoculating plant growth-promoting bacteria under chromium stress. Under chromium stress the diameter of different tissues of root and shoot was decreased that had not been reported.

Based on these results we can conclude that along with many other mechanisms (producing phytohormones, fixing atmospheric nitrogen, phosphate solubilization, and production of siderphores) involved for growth stimulation of sunflower seedlings, it was obvious that bacterial strains promoted plant growth by decreasing bioavailability of toxic Cr(VI).

Received April 18, 2004; accepted March 30, 2005.

LITERATURE CITED

Afrasayab S, Zeb A, Hasnain S (2001) Variable effects of mono and mixed culture bacterial inoculations on *Triticum aestivum* grown under salt stress. Pak J Microbiol 1: 29-41

Ben-Effraim I, Gadd AE, Cohen P, Reymond P, Pilet PE (1990) The effect of 4-chlororesorcinol on the endogenous level of IAA, ABA and oxidative enzymes in cutting. Plant Reg 9: 97-106

Benova D, Hadjidekova V, Hristova R, Nikolova T, Boulanova M, Georgieva I, Grigorova M, Popov T, Panev T, Georgieva R, Natarajan AT, Darroudi F, Nilsson R (2002) Cytogenetic effects of hexavalent chromium in Bulgarian chromium platers. Mutat Res 514: 29-38

Burd GI, Dixon G, Glick BR (1998) A plant growth-promoting bacterium that decreases nickel toxicity in seedlings. Appl Environ Microbiol 64: 3663-3668

Burd GI, Dixon G, Glick BR (2000) Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46: 237-245

Camargo FAO, Bento FM, Okeke BC, Frankenberger WT (2004) Hexavalent chromium reduction by an actinomycete, arthrobacter crystalopoietes ES 32. Biol Trace Elem Res 97: 183-194

Clesceri SL, Greenberg AE, Eaton AD (1998) Standard methods for the examination of water and waste water, Ed 20, American Public Health Association. Washington D.C., pp 3-66

Dushenkov V, Kumar P, Motto H, Raskin I (1995) Rhizofilteration, the use of plants to remove heavy metals from aqueous streams. Environ Sci Technol 29: 1239-1245

Ehrlich HL (1997) Microbes and metals. Appl Microbiol Biotechnol 48: 687-692

Hasnain S, Sabri AN (1997) Growth stimulation of *Triticum aestivum* seedlings under Cr-stresses by Non-Rhizospheric *Pseudomonas* strains. Environ Pollut 97: 265-273

Hewitt EJ (1963) Minerals nutrition of plants in culture media, *In* FC Steward, ed, Plant Physiology. Academic Press, New York, pp 99-137

Hoflich G, Metz R (1997) Interaction of plant microorganism-association in heavy metal containing soils from sewage farms. Bodenkultur 48: 238-247

Humphries EC (1958) Mineral component and ash analysis, *In* K Paech, MY Traley, eds, Modern Methods of Plant Analysis. Springer Verlag, Berlin

Iqbal J, Rafique N (1986) Toxic effect of $BaCl_2$ on germination, early seedling growth, soluble proteins and acid phosphatase in *Zea mays* L. Pak J Bot 19: 1-8

Kapulnik Y (1996) Plant growth promotion by rhizobacteria, *In* Y Waisel, A Eshel, U Kafkazi, eds, Plant Roots: The Hidden Half. Marcel Dekker, New York, pp 769-781

Kastori RM, Petrovic M, Petrovic N (1992) Effect of excess lead, cadmium, copper and zinc on water relation in sunflower. J Plant Nutr 15: 2427-2439

Mabbett AN, Lloyd JR, Macaskie LE (2002) Effect of complexing agents on reduction of Cr(VI) by *Desulfovibrio vulgaris* ATCC 29579. Biotechnol Bioeng 79: 389-397

Macaskie LE (1995) Use of biological processes for the removal of heavy metal and *organophosphorous* compounds from aqueous solution, *In* N Ahmad, M Ishfaq, OB Khan, F Sarwar, eds, Biotechnology for Environment and Agriculture. BCC and T Press, University of Karachi, Karachi, pp 127-142

Mehmood U, Sabri AN, Hasnain S (1998) Involvement of plasmid associated with chromium resistant pseudomonas strains in stimulation of *Triticum aestivum* seedlings grown under cr-stresses. Biologia 1: 204-217

Montgomery DM, Dean AC, Macaskie LE (1995) Phosphatase production and activity in *Citrobacter freundii* and a naturally occurring, heavy-metal-accumulating *Citrobacter* sp. Microbiol 141: 2433-2441

Mutaftchier S, Macaya A, Prat R, Deviller P, Colberg R (1993) Early effects of plant cell wall fragment on plant cell growth. Plant Physiol Biochem 31: 459-467

Nichols PB, Cough JD, Al-Hamdani SH (2000) Selected physiological responses of *Salvinia minima* to different chromium concentrations. Aquatic Bot 68: 313-319

Proctor DM, Otani JM, Finley BL, Paustenbach DJ, Bland JA, Speizer N, Sargent EV (2002) Is hexavalent chromium carcinogenic via ingestion? A weight-of-evidence review. J Toxicol Environ Hlth Part A 65: 701-746

Saleh MT, Belisle JT (2000) Secreation of acid phosphatase (Sap M) by *Mycobacterium tuberclosis* that is similar to eukaryotic acid phosphatase. J Bacteriol 182: 6850-6853

Sanderson JB (1994) Biological Microtechnique. RMS Handbook 28, BIOS Scientific Publishers, Oxford

Stoychev D, Stefanov P, Nicolova D, Valov I, Marinova T (2002) Chemical composition and corrosion resistance of passive chromate films formed on stainless steels 316 L and 1.4301. Mat Chem Phys 73: 252-258

Wyss M, Pasamontes L, Rémy R, Kohler J, Kusznir E, Gadient M, Müller F, van Loon APGM (1998) Comparison of the thermostability properties of three acid phosphatases from molds: *Aspergillus fumigatus* phytase, *A. niger* phytase, and *A. niger* pH 2.5 acid phosphatase. Appl Environ Microbiol 64: 4446-4451