GROWTH PROMOTING ATTRIBUTES OF CHROMIUM (VI) RESISTANT *BACILLUS* STRAINS FOR *TRITICUM AESTIVUM* L.

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ABSTRACT

Hexavalent chromium (Cr) is carcinogenic, mutagenic and causes many health hazards. Some bacteria are capable of resisting high concentrations of hexavalent Cr by converting it into trivalent form, which is non-toxic. The purpose of this study was to isolate *Bacillus* strains resistant to Cr (VI) and also proficient to promote plant growth. The strains *B. rhizosphaerae* KAC5, *B. clausii* KSC6 and *B. cereus* SLC9 were capable to grow at 500 to 4000 µg/ml Cr (VI). The strains showed growth over wide range of pH (pH 5-11), temperature (32-55ºC) and sodium chloride concentration (up to 5% (w/v). The *Bacillus* strains showed 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity and produced indole-3-acetic acid (IAA) up to 152 µg/ml. The strains were also positive for phosphate solubilization and siderophore synthesis. Seeds of *Triticum aestivum* L. treated with *Bacillus* strains were grown in pots having Cr (VI) concentrations 0, 200 and 500 mg/kg of soil. The *Bacillus* strains significantly improved the growth of *T. aestivum* by increasing its shoot length, number of tillers, spike length, number of spikelets, number of grains and seed weight. Hence, the Cr (VI) resistant *Bacillus* strains showed potential to be used for the growth promotion of plants under chromium stress.

Keywords: ACC-deaminase activity, Bacterial auxin production, Chromium resistant bacteria, *Bacillus*, Cr (VI) reduction, plant growth promoting bacteria.

INTRODUCTION

Chromium (Cr) is a natural transition metal having atomic number 24 and atomic mass 51.996. Chromium is a solid, steel-gray, gleaming and brittle metal. It commonly exists as Cr (III) and (VI) (Xu *et al*., 2012). Cr (III) makes compounds like oxides, hydroxides and sulfates. It usually occurs bound to organic matter of soil and aquatic surroundings. It does not exist freely that's why less toxic (Sayel *et al*., 2012). Cr (III) is also an important micronutrient, important for the mammals and yeasts in normal carbohydrate metabolism. Cr (III) involvement is observed in protein tertiary structure as well as in configuration of DNA and RNA of cell (Focardi *et al*., 2013). Chromium (VI) is compound with oxygen as chromate and dichromate. Cr (VI) is more soluble and penetrable, enters into food chain via plant roots absorption (Sayel *et al*., 2014). It is 1000-fold cytotoxic and mutagenic as compared to trivalent Cr (Dhal *et al*., 2010). The release of Cr (VI) as liquid effluent during the last 15 years is about 31000 – 72000 kg (Wang *et al*., 2015). Recently, the pollution status of heavy metals, especially, chromium has gained great concern in Pakistan. For instance, ground water samples from different areas recorded up to 9.8 mg /l chromium (Waseem *et al.,* 2014). Moreover, a great variety of chromium resistant rhizospheric or endophytic bacteria were reported from the tannery effluent contaminated soils of city Kasur, Pakistan (Khan *et al.,* 2015).

Chromium (VI) compounds are carcinogenic (Kathiravan *et al*., 2010). Human health problems related to chromium toxicity in the body include lung cancer, liver disorders, low number of platelets and kidney failure (Saharan and Nehra, 2011; Monachese *et al*., 2012). Chromium toxicity also causes disorders in plants such as chromosomal aberrations, disruption of mitosis, reduced photosynthesis or DNA damange (Ahemad, 2015; Schiff *et al.*, 2016). However, few crops are unaffected at Cr concentration below the 4.0 $x10^{-4}$ µM (Riaz *et al*., 2010). Chromium is noxious to higher plants at 0.1 mM /kg dry mass (Pang *et al*., 2011). Chromium (VI) is a resilient oxidizing agent and itself converted into Cr (III) in the presence of organic matter. Conversely, high Cr (VI) levels might astounded the reducing capability of the organic matter of surroundings and consequently persist in hexavalent form (Saha *et al*., 2011; Chatterjee *et al.,* 2012). The conversion of Cr (VI) in to Cr (III) reduces its mobility and toxicity towards environment (Liu *et al*., 2012). The physicochemical methods for conversion from hexavalent to trivalent Cr are very costly and hazardous to environment. Therefore, such strategies cannot be applied at commercial level (Cheng *et al*., 2012).

Bacterial enzymes and cells adsorbed or encapsulated in various polymeric substances have been used effectively for chromium reduction (Pal *et al.,* 2013). Bacterial hexavalent chromium reduction process comprises three steps that included attachment of chromium to cell surface, entry into the cell and finally

its reduction inside the cell (Bahafid *et al*., 2011). Intake via bacterial cell is done passively and then, accumulates slowly upon cell energy (Joutey *et al*., 2015). Some rhizobacteria contain beneficial traits for growth promotion of host plants growing in metal contaminated soils. Metal resistant rhizobacteria can enhance plant growth by employing different mechanisms. It included 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, biosynthesis of indole-3-acetic acid (IAA) and siderophores production (Figueiredo and Pereira, 2010; Khan *et al.,* 2015). Microorganisms capable of resisting toxic heavy metals along with plant growth promotion potential may increase the accessibility of heavy metals in their non-toxic form for plants (Yu *et al*., 2011).

The genus *Bacillus* embraces metabolically versatile, spore-former, and ubiquitous bacteria. *Bacillus* strains isolated from wastewater, sediments, and soils exhibit plant growth promoting as well as Cr reducing traits (Chaturvedi, 2011). *B. cereus* remains viable and functional in higher concentration of Cr (Adeel *et al*., 2012). It is a spore former and withstands the stresses of drought, high temperature, high pH, salt, pollutants and toxins of the environment. It overcomes the stresses due to ACC-deaminase activity, which interacts with IAA and enhances the plant growth. Therefore, *Bacillus* strains are best for the growth enhancement of the crops in heavily metal loaded soils (Khalimi *et al*., 2012). In present study, chromium resistant bacteria capable of metal remediation were isolated and screened biochemically or physiologically and assessed for various plant growth promoting attributes. Influence of bacterial strains on the growth promotion of *Triticum aestivum* L. in Cr (VI) stress was also evaluated in pot trials.

MATERIALS AND METHODS

Isolation and characterization of Cr (VI) resistant bacterial isolates: Soil samples were collected from effluent discharge sites of different industries (Lahore, Sialkot and Kasur, Pakistan) that were heavily contaminated with chromium. Samples were collected in sterile glass jars following the protocol of Beneduzi *et al*. (2008). One gram of soil sample was added to 9 ml sterilized distilled water, thoroughly mixed and serially diluted to from 10^{-1} to 10^{-9} dilutions. These serial dilutions were spread on Luria-Bertani agar (L-agar) plates amended with 200 mg/litre of K_2CrO_4 as described by Kumar *et al*. (2008). Isolated strains were purified by quadrant streaking. Selected strains were then characterized morphologically and biochemically.

16S rRNA gene sequencing: The genomic DNA from bacterial strains was extracted with Mini Prep Kit (Favor Prep Biotech Corp. Taiwan) and amplified for 16S rDNA by PCR. The reaction mix contained 25 µl PCR Master Mix (Thermo Fisher Scientific Inc. USA.), 7 µl DNA templates, 2 µl each of forward and reverse primer and 14 µl nuclease free water. The reaction mixture was incubated in thermal cycler PCR (GeneAmp PCR system 9700, Applied Biosystems, USA) at 94ºC for five minutes. Afterwards, thirty cycles of denaturation, annealing and extension, of 45 sec, 65 sec and 70 sec were carried out at temperatures, 94ºC, 60ºC and 72ºC, respectively. Amplified PCR products were purified by using FavorGen Gel Extraction Kit (Favor Prep Biotech Corp. Taiwan). Purified samples were sequenced by using 27f (AGAGTTTGATCCTGGCTCAG) and 1492r (TACGGTTACCTTGTTACGACTT) primers by sending samples to First Base Laboratories, Singapore.

Chromium resistance and reduction: To determine the Cr resistance level of the strains, they were grown in Luria-Bertani broth (L-broth) amended with varying concentrations of Cr (200 to 4000 mg/liter). Bacterial growth was observed as culture density at 600 nm. The growth of chromium resistant *Bacillus* strains was also evaluated at different temperatures, pH and salt concentrations. Effect of temperature was evaluated by growing strains at 32ºC, 37ºC, 45ºC and 55ºC. Effect of pH was determined by growing strains at pH 4, 7, 9, and 11. Similarly, effect of sodium chloride on the growth of strains was evaluated in L-broth by using different concentrations of NaCl. Chromium (VI) reduction capability of bacterial strains was determined with diphenylcarbazide (DPC) assay (APHA, 1989).

Plant growth promoting attributes: 1-Aminocyclopropane-1-carboxylate (ACC) deaminase activity was checked following Li *et al*. (2011). Indole-3-acetic acid (IAA) synthesis was estimated following the method of Bric *et al*. (1991). The bacterial strains were grown in Luria-Bertani broth (l-broth) supplemented with different L-tryptophan concentrations (700, 1000 and 1500 µg/ml). For each strain, three replicates were placed for comparison. After incubation, 2 ml supernatant was taken and mixed with 0.1 ml of 10 mM H_3PO_4 and 4 ml of Salkowski's reagent. The red color developed after 25 min incubation was recorded and optical densities were measured at 530 nm (Rajkumar and Freitas, 2008). For phosphate solublization, the strains were grown on modified NBRIP medium (Nautiyal, 1999) with 0.5% of tri-calcium phosphate. Bacterial strains were incubated for 7 days at 30°C. The clear zones around the growth were measured. For siderophore assay, strains were grown on the Chrome Azurol S agar medium that was modified by Alexender and Zuberer (1991). Qualitative hydrogen cyanide (HCN) synthesis test was performed according to the method of Lorck (1948).

Pot trials: Certified seeds of *Triticum aestivum* L. var. Galaxy 2013 were obtained from Punjab Seed Corporation, Lahore, Pakistan. Healthy seeds in good physical shape were surface sterilized with $HgCl_2$ (0.1%)

for 2-3 min with constant shaking. Then, seeds were rinsed twice with sterile distilled water. Afterwards, seeds were incubated in bacterial cultures maintained at 10^7 CFU/ml for 30 min. For pot trials, 10 kg sieved, oven dried soil was poured in each pot. Pot soil was stressed with 0, 200 and 500 mg/kg concentrations of Cr metal as K_2CrO_4. Complete pot experiment was conducted in triplicates. For each strain, pot soil was amended with 0, 200 and 500 mg/kg of K_2CrO_4. Control seeds were treated with K_2CrO_4 without bacterial inoculations. Ten seeds were sown in each pot. After three months, when seedlings attained height but spikes were not formed, three plants from each pot were taken for fresh and dry weight. Same day further thinning of plants was done, leaving 3 plants per pot. At full maturity, shoot length, tillers per plant, spike length, spikelets per spike and weight of 100 grains from each treatment were documented.

Statistical analysis: The data obtained in all the experiments were evaluated statistically for one-way descriptive ANOVA by using SPSS 20.0 Software and mean values were compared by using Duncan's multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Characterization and identification of bacterial strains: Bacteria were isolated from different metal contaminated soil and water sites, capable of reducing chromium and making soil worthy for living being. Because of stress these bacteria may also have developed many plant growth promoting traits. Therefore, such bacteria are most suitable to be used as plant growth promoting bacteria (PGPB) in heavy metal contaminated soils (Mandal *et al*., 2011). The isolated strains were screened on the basis of their ability to resist chromium. Three strains KAC5, KSC6 and SLC9 were finally selected for further study. The strains were gram positive rods, spore former and non-capsulated. Strains formed irregular, flat and white colored colonies. The strains showed positive results for catalase, citrate, nitrate reduction, glucose utilization and hydrolysis of gelatin, casein and starch. The 16S rRNA gene sequences of the strains were searched for homologies using NCBI nucleotide BLAST search program (BLASTN). According to BLASTN the sequences of strains KAC5, KSC6 and SLC9 were found homologous to the strains *Bacillus rhizosphaerae, B. clausii*, *B. cereus.* Finally, strains were identified as *B. rhizosphaerae* KAC5, *B. clausii* KSC6 and *B. cereus* SLC9. The nucleotide sequences were submitted to GenBank and allotted accession numbers from KX417229 to KX417231. Chromium resistant bacteria can colonize the rhizspheric soils inhabiting by natural plant settings. For instance, great diversity of rhizospheric or endophytic bacteria from the root system of *Prosopis juliflora* was reported from chromium contaminated soils (Khan *et al.,* 2015).

Effect of physical and chemical factors on bacterial growth: The *Bacillus* strains showed growth in L-broth medium supplemented with K_2CrO_4 concentration up to 4000 µg/ml with KAC5 and KSC6 exhibiting slightly better growth than SLC9 (Fig. 1). The strains showed good growth up to 1000 µg/ml, after this gradual decline was started. Bacterial Cr (VI) resistance of up to 2500 µg/ml has been reported by Camargo *et al.* (2003). Cr-resistant bacteria isolated from plant endosphere and rhizosphere were able to tolerate 3000 µg/ml of chromium (Khan *et al*., 2015). The isolated *Bacillus* strains were capable of growth at mesophilic as well as thermophilic temperatures. All the strains showed good growth at temperature range 32-55ºC. The KAC5 and KSC6 showed optimum growth at 32ºC while SLC9 exhibited optimum growth at 45ºC (Fig. 2). Although 55ºC was not suitable, yet SLC9 displayed better growth at 55 as compared to 32ºC. Usually *Bacillus* strains are mesophiles but occasionally behave as hypothermophiles, thermophiles and hyperthermophiles (Panda *et al.,* 2013). All the three strains grew well at pH 5-11. The *Bacillus* strains KSC6 and SLC9 gave best growth at alkaline pH 9, while KAC5 exhibited optimum growth at pH 5 (Fig. 3). The growth of *Bacillus* strains was noted from 0% to 5% NaCl concentrations (Fig. 4). The strain SLC9 showed good response towards salt concentrations up to 1.5%. Such strains can be used for the treatment of contaminated saline soil as these are capable of growing in high salt concentrations (Sagar *et al*., 2012). The salt tolerance by bacteria is a positive trait in plant growth promotion (Khan *et al*., 2013). The *Bacillus* strains were grown in the absence and presence of chromium for the determination of growth curves. There was slight difference in the growth in the absence and presence of Cr (VI). The growth in the presence of chromium was better than growth in the absence of chromium (Fig. 5). Metal stress increased the efficiency of metal resistant bacteria (Pal *et al*., 2013). The chromium resistant bacteria resist chromium by converting its toxic hexavalent form into its non-toxic trivalent form. The amount of chromium (VI) reduced was 60%, 70% and 67% by strains KAC5, KSC6 and SLC9, respectively after 24 hours at Cr(VI) concentration of 500 µg/ml (Fig. 6). *Bacillus* sp. strain CBS-4 showed more than 90% Cr (VI) reduction with initial Cr (VI) concentration of 100 µg/ml after 144 hours (Dhal *et al.*, 2010; Waseem *et al.,* 2014).

Plant growth promoting attributes: The Cr (VI) resistant bacterial strains were tested for different plant growth promoting characters (Table 1). Each strain was positive for ACC-deaminase activity and strain KAC5 displayed the best activity that was 260.2 nmol/ml (data not shown). ACC deaminase activity is a vital role of

plant growth stimulating bacteria. It results in the decrease of stress ethylene in plants by converting it into ACC and then utilization of ACC as sole nitrogen source (Li *et al*., 2011; Sessitsch *et al.,* 2013). ACC-deaminase activity is also very beneficial for plant growth as it inhibits sagging of flowers, form nodules and increase root tips growth (Gamalero and Glick, 2015). All the three strains synthesized good amount of IAA when medium was added with different concentrations of L-tryptophan. At 700 µg/ml and 1500 µg/ml L-tryptophan, *B. cereus* SLC9 showed good IAA production while at 1000 µg/ml *B. rhizosphaerae* KAC5 showed good IAA production (Table 2). There was not much difference in IAA production at 1000 and 1500 µg/ml tryptophan concentration for all strains. So the increase of tryptophan level up-to a specific amount increased the IAA synthesis and above this level further increase of tryptophan amount did not increase IAA production remarkably (Won *et al*., 2011). IAA synthesis increases the surface area of root and nutrient uptake by plants (Raheem *et al.,* 2018). The strains KAC5, KSC6 and SLC9 solubilized phosphate on modified NBRIP medium and showed hollow zones of 7 mm, 8 mm and 6 mm, respectively (data not shown). All the strains also showed positive results for siderophore synthesis on Chrome Azurol S Agar plates but HCN production was only shown by strain SLC9. Most of the Cr-resistant rhizo- and endophytic strains isolated from *Prosopis juliflora* also displayed one or more plant growth promoting characteristics (Khan *et al.*, 2015).

Pot trials: Bacterization of wheat seeds with *B. rhizosphaerae* KAC5, *B. clausii* KSC6 and *B. cereus* SLC9 showed significant growth increase over respective un-inoculated controls (Table 3, 4). Inhibitory effect on plant growth was recorded when soil was amended with chromium. For example, when amount of chromium was increased from 200 mg/kg to 500 mg/kg, there was more than 13% decrease in plant length while in case of control decrease in growth at 200 and 500 mg/kg chromium was 20% and 17%, respectively (Table 3). However, seed treatment with chromium resistant *Bacillus* strains significantly mitigated the negative impacts of chromium on plant growth. For instance, at 200 mg/kg of chromium, *B. cereus* SLC9 and *B. clausii* KSC6 showed 28% and 23% increases for shoot length, respectively, over chromium amended control. At 500 mg/kg, bacterial inoculations showed up to 36% increase with *B. cereus* SLC9. For number of tillers, 20% improvement was recorded with *B. rhizosphaerae* KAC5 and *B. clausii* KSC6, over control at 200 mg/kg chromium. Similarly, at higher concentration i.e. 500 mg/kg, *B. clausii* KSC6 witnessed 50% improvement, over control. For spike length, up to 19% and 43% increase was recorded with *B. cereus* SLC9 at 200 and 500 mg/kg chromium, respectively, over control. For number of spikelets, *B. rhizosphaerae* KAC5 was the most effective to record up to 14% increase in chromium amended soils. For number of seeds per spike, *B. rhizosphaerae* KAC5 was the most promising to show 14% increase at 500 mg/kg chromium. For 100 seed weight, statistically marginal improvements were observed as compared to respective control treatments. In present study, the maximum chromium concentration used in pot trials was 500 mg/kg of soil. In one study, chromium amendments in soil ranged from 20-70 µg/kg of soil (Khan *et al*., 2013) and higher concentration was 136 µg/kg used by Wani *et al*. (2007). Wang et al. (2012) demonstrated the growth enhancement of plants in chromium amended soils after bacterial inoculations. The stimulatory effects of Cr resistant *Bacillus* strains on wheat growth might be due to the their ability to detoxify toxic Cr (VI) as well as plant growth promoting activities (Khan *et al.*, 2015).

For fresh biomass, more than one fold increase was recorded with bacterial inoculations when compared with un-inoculated control. However, chromium amendments significantly reduced the biomass of plants as compared to control (Table 4). On the other hand, inoculation of wheat seeds with chromium resistant *Bacillus* strains significantly lowered the toxicity of chromium as evident from up to 4 and 10 fold increase in fresh biomass at 200 and 500 mg/ kg chromium, respectively, over un-inoculated control. For dry biomass, 10 fold increase was also recorded with *B. cereus* SLC9 at higher concentration of chromium. Khan *et al.* (2015) reported the improvement in seed germination, root or shoot length and increase in plant biomass with chromium resistant bacteria.

Finally, it can be concluded that *Bacillus* strains have the ability to tolerate high levels of chromium i.e., up to 4000 µg/ml. These strains were able to grow over wide ranges of temperature, pH and NaCl. *Bacillus* strains exhibited plant growth promoting properties such as ACC-deaminase activity, IAA production, phosphate solubilization and siderophore synthesis. The inoculation of *T. aestivum* seeds with chromium (VI) resistant *Bacillus* strains significantly increased plant growth parameters in chromium amended soils. Hence, chromium (VI) resistant *Bacillus* strains can be used to promote the growth of plants in chromium contaminated soils.

Table 1. Plant growth promoting attributes of Cr (VI) resistant *Bacillus* strains.

Strains	Plant growth promoting attributes				
	ACC deaminase activity	IAA production	Phosphate solubilization	Siderophore synthesis	HCN production
KAC5	+	+	+	+	-
KSC6	+	+	+	+	-
SLC9	+	+	+	+	+

Table 2. IAA synthesis by *Bacillus* strains at different concentrations of L-tryptophan.

Cr (VI) resistant strains	Tryptophan µg/ml		
	700	**1000**	**1500**
KAC 5	44.36^{a}	143.57^{f}	136.06^{e}
KSC6	48.35^{b}	135.29^{e}	145.87^{f}
SLC9	56.93^{c}	130.08^{d}	152.62^{g}

"Each value is mean of three replicates. Different letters on values within each column specify significant difference between treatments using Duncan's multiple range test ($P \leq 0.05$)"

Table 3. Effect of *Bacillus* strains on vegetative and yield parameters of wheat in chromium amended soils

Strains with metal mg/kg soil	Shoot length (cm)	Number of tillers	Spike Length (SpL) cm	No of spikelets (SpN)	Number of seeds/spike (SN)	Weight of 100 seeds (SW) g
KAC5 0	91.44^{e}	4.00^{c-e}	15.41^{f}	17.67^{d}	52.00^{e}	4.30^{d-f}
KAC5 200	74.93^{d}	4.00^{c-e}	13.29^{de}	17.00^{cd}	50.00^{d}	4.20^{de}
KAC5 500	66.46^{c}	2.33^{ab}	12.70^{cd}	16.00^{bc}	48.00^{b-d}	4.00^{bc}
KSC6 0	91.44^{e}	4.67^{e}	14.82^{f}	17.67^{d}	49.00^{d}	4.30^{d-f}
KSC6 200	74.93^{d}	4.00^{c-e}	11.85^{bc}	17.00^{cd}	48.00^{b-d}	4.00^{bc}
KSC6 500	66.46^{c}	3.00^{a-c}	11.09^{b}	15.00^{ab}	42.00^{a}	3.87^{ab}
SLC9 0	92.71^{e}	4.33^{de}	14.90^{f}	17.67^{d}	52.67^{e}	4.43^{f}
SLC9 200	78.32^{d}	3.67^{c-e}	14.39^{ef}	16.67^{cd}	49.33^{d}	4.37^{ef}
SLC9 500	68.58^{c}	2.00^{a}	12.70^{cd}	15.00^{ab}	46.00^{b}	4.20^{de}
Control 0	76.62^{d}	3.67^{c-e}	12.70^{cd}	16.33^{b-d}	48.67^{cd}	4.13^{cd}
Control 200	60.96^{b}	3.33^{b-d}	12.11^{b-d}	15.00^{ab}	46.67^{bc}	3.97^{a-c}
Control 500	50.38^{a}	2.00^{a}	8.89^{a}	14.00^{a}	42.00^{a}	3.80^{a}

"Each value is mean 9 plants. Different letters on values within each column specify significant difference between treatments using Duncan's multiple range test ($P \leq 0.05$)"

Table 4. Effect of *Bacillus* strains on fresh weight and dry weight of wheat plants in chromium amended soils.

Strains with metal mg/kg soil	Fresh plant weight (g)	Dry plant weight (g)
KAC5 0	8.40^{h}	2.10^{h}
KAC5 200	5.29^{f}	1.32^{f}
KAC5 500	2.59^{c}	0.65^{c}
KSC6 0	7.08^{g}	1.77^{g}
KSC6 200	4.14^{e}	1.03^{e}
KSC6 500	2.65^{c}	0.66^{c}
SLC9 0	7.05^{g}	1.76^{g}
SLC9 200	5.00^{f}	1.25^{f}
SLC9 500	3.25^{d}	0.81^{d}
Control 0	3.17^{d}	0.79^{d}
Control 200	1.06^{b}	0.26^{b}
Control 500	0.27^{a}	0.07^{a}

"Each value is mean of 9 plants. Different letters on values within each column specify significant difference between treatments using Duncan's multiple range test ($P \leq 0.05$)"

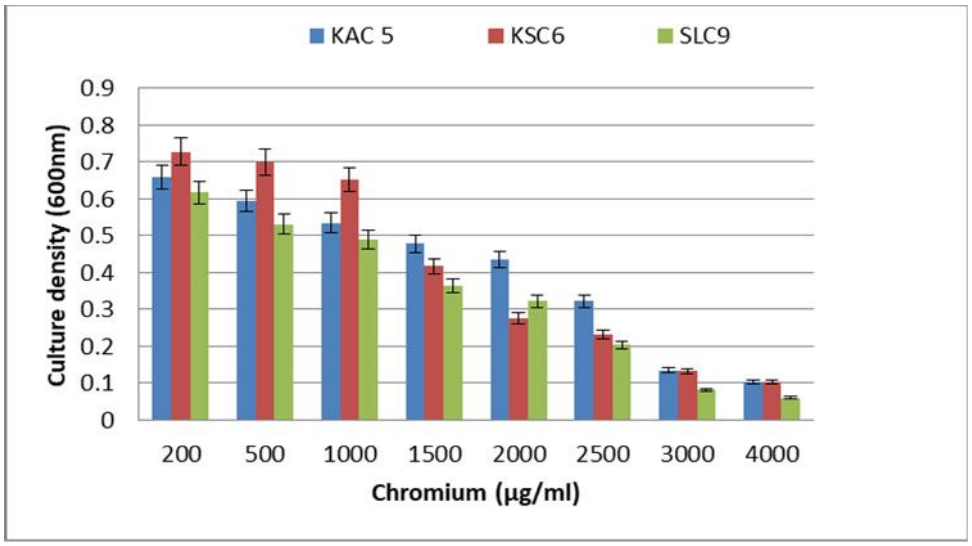

Figure 1. Effect of different Cr (VI) concentrations on the growth of Cr (VI) resistant *Bacillus* strains.

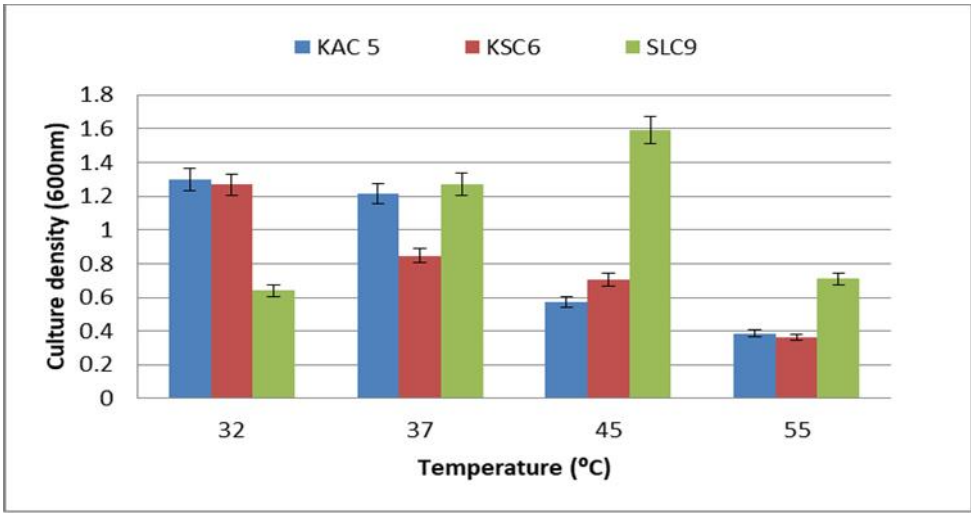

Figure 2. Effect of varying temperatures on the growth of Cr (VI) resistant *Bacillus* strains.

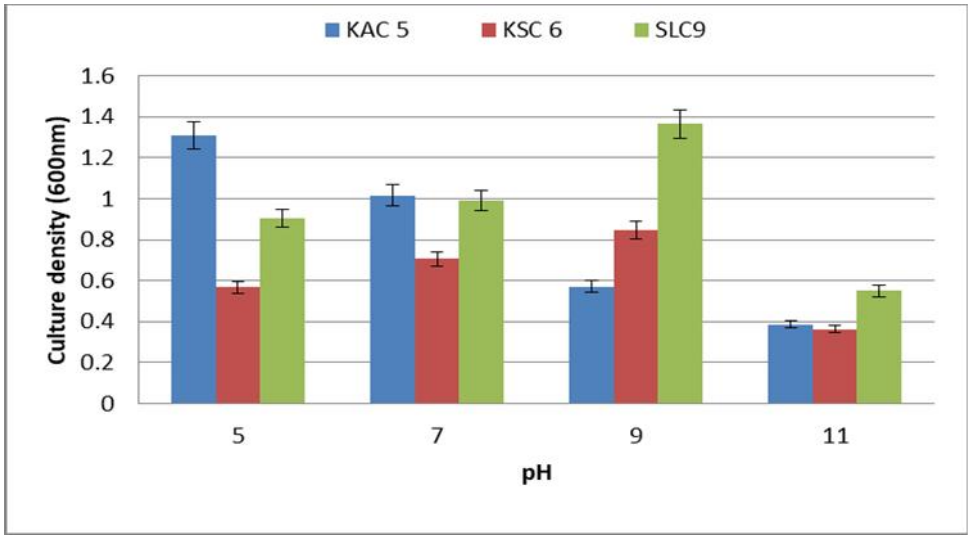

Figure 3. Effect of varying pH on the growth of Cr (VI) resistant *Bacillus* strains.

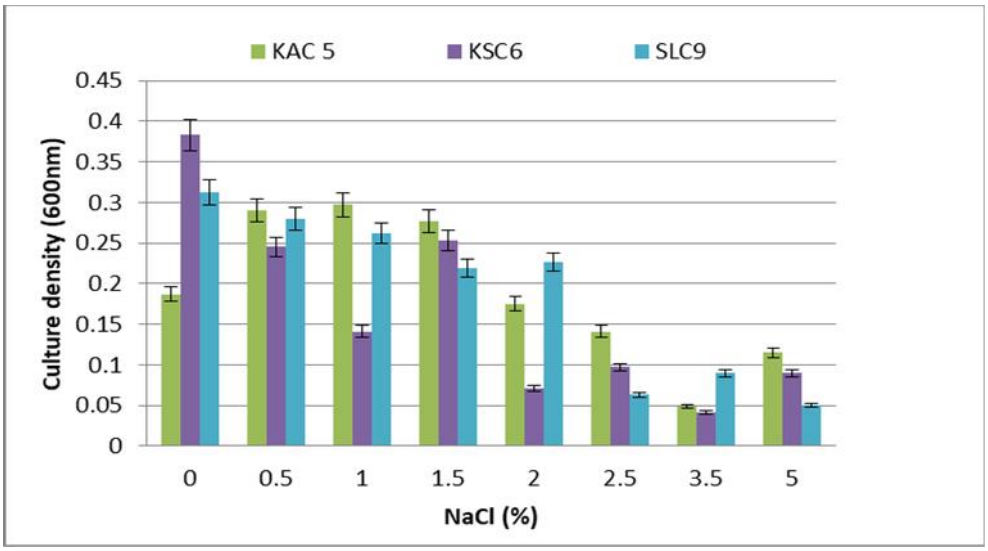

Figure 4. Effect of NaCl on the growth of Cr (VI) resistant *Bacillus* strains.

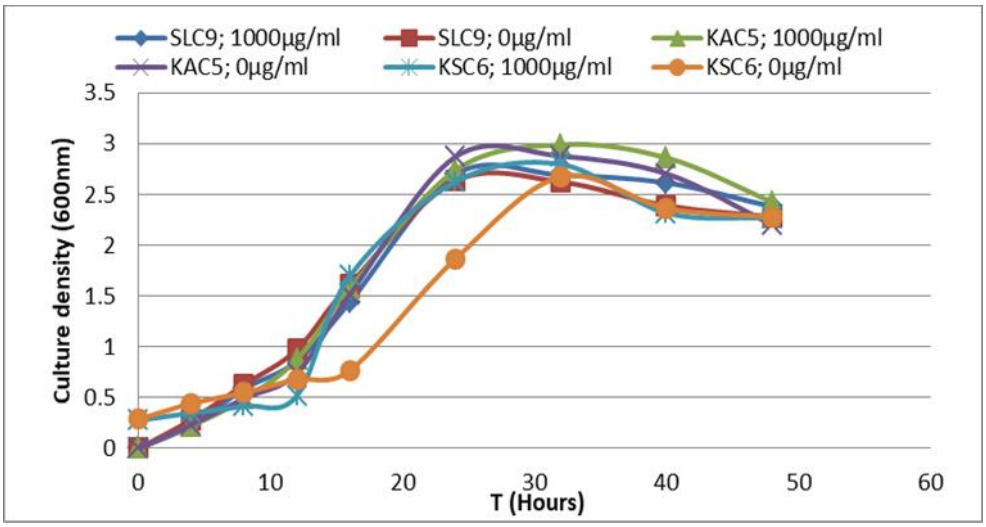

Figure 5. Growth curves of Cr (VI) resistant *Bacillus* strains in absence and presence of chromium (VI).

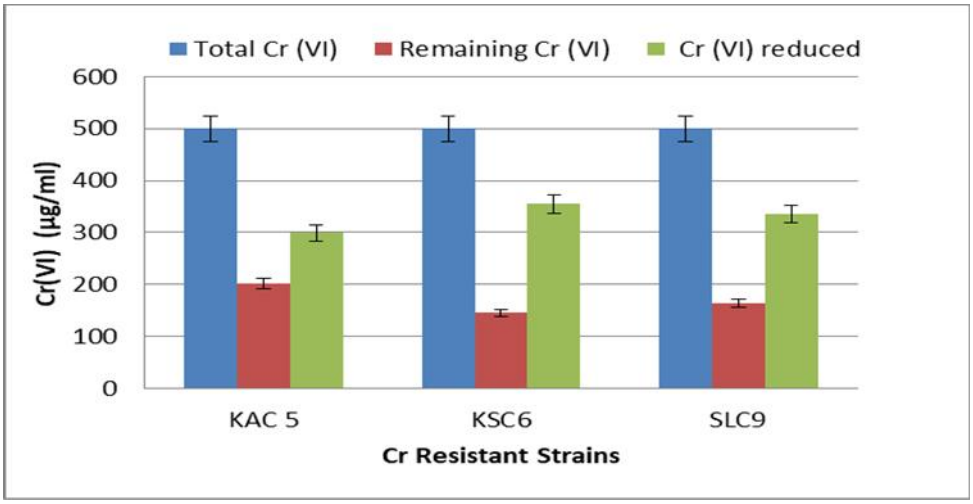

Figure 6. Chromium (VI) reduction by *Bacillus* strains at initial Cr (VI) concentration of 500 µg/ml.

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