



Original article

Multiple metal resistance and Cr⁶⁺ reduction by bacterium, *Staphylococcus sciuri* A-HS1, isolated from untreated tannery effluent

Amina Elahi, Abdul Rehman*

Department of Microbiology and Molecular Genetics, University of the Punjab, New Campus, Lahore 54590, Pakistan

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ABSTRACT

A chromium-resistant bacterium was isolated indigenously from tannery effluent and identified as *Staphylococcus sciuri*. The optimum growth was observed at 37 °C and pH 7. *S. sciuri* was able to resist Cr⁶⁺ (25 mM) and other heavy metals such as As²⁺ (19 mM), Pb²⁺ (18.5 mM), Zn²⁺ (17 mM), Cu²⁺ (2.5 mM), Cd²⁺ (3 mM), and Ni²⁺ (1.5 mM). Biochemical characterization of chromate reductase revealed its optimal pH as 8.0 and temperature as 40 °C. The enzyme activity was stimulated only by Mg²⁺ among other heavy metals tested. *S. sciuri* showed chromium biosorption proficiency (q) after 2, 4, 6 and 8 days as 42, 73, 85 and 31 mM/g, respectively. Cr⁶⁺ did not stimulate activities of APOX, SOD and CAT significantly, however, only POX showed marked production (86%). Also, enhanced production of glutathione and other non-protein thiols significantly combat metal induced oxidative stress. Pilot study verified that *S. sciuri* was able to remove 93% Cr⁶⁺ from tannery effluent within 6 days of incubation. Thus, *S. sciuri* could act as a potential candidate for the bioremediation of hexavalent chromium contaminated environmental sites.

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1. Introduction

Advancement in technology and industrial globalization led to indiscriminate emission of hazardous industrial wastes into the biosphere, consequently inflict serious damage to the environment. The industrial effluents usually consist of a huge amount of various toxic heavy metals at an elevated concentration, when discharged directly into the environment without any prior treatment; severely contaminate the land and fresh water bodies (Nissim et al., 2018).

Anthropogenic inputs of chromium has been increased due to its widespread use in industries like electroplating, tanning of hides and skins, resistant stainless steel alloys production, textile dye productions (Cheunga and Gu, 2007), nuclear coolants (i.e., biocide) and mining activities. Thus, huge amount of Cr-containing wastes has been released in the industrial effluents that contaminate the ecosystem. When gets in water bodies, Cr is

toxic to the plants and animals due to its mutagenic, carcinogenic, and teratogenic effects.

Tanning industry generate huge varieties of toxins such as salinity, inorganic and organic load (chemical and biological oxygen demand), dissolved, suspended solids, specific pollutants (chromium, sulfide, sodium, chloride and other salt residues) and heavy metals etc. (Katz and Salem, 1994; Zahoor and Rehman, 2009). Tanning process utilizes huge quantities of water out of which 90% water get discarded as effluent. Also solid and gaseous waste byproducts generated during leather processing contaminate the environment. Chrome tanning procedure discharges at least 40% unused chromium salts in the final effluents, posing a serious threats to the environment (Leghouichi et al., 2009; Garg et al., 2012).

As a transition metal, Cr exists in various valence states i.e., –2 to +6, with the hexavalent (Cr⁶⁺) and trivalent (Cr³⁺) being the predominant chromium species in the natural aquifers and municipal wastewater rich in organic contaminants, respectively (Cheunga and Gu, 2007). Dominancy of Cr⁶⁺ in the aquifer is due to its high solubility in water in the full pH range, making it bioavailable in the ecosystem. In contrast, Cr³⁺ displays a high affinity for organics resulting in the formation of complexes that precipitate as amorphous hydroxide (Sawyer et al., 1994). Because of its persistence in the environment, anthropogenic release of Cr⁶⁺ is a matter of concern (Katz and Salem, 1994; Cheunga and Gu, 2007).

* Corresponding author.

E-mail address: rehman.mmg@pu.edu.pk (A. Rehman).

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Heavy metals, non-degradable in nature, easily get accumulated in the environment without limitations, hence removing them mechanically is not only an expensive approach but also time consuming. Thus, biological removal of heavy metals from the effluents has been utilized for the bioremediation purposes as it primarily depends on the ability of microbes to uptake and/or reduce the metal (Mala et al., 2015).

Ability of microorganisms to survive and proliferate within the vicinity of excruciating toxic metals has helped the scientists to develop several strategies for the bioremediation of hazardously polluted environments. Thus, the discovery of chromate reducing microorganisms proved to be a potential candidate for *in situ* and on-site bioremediation approaches (Garg et al., 2012; Mangaiyarkarasi and Geetharamani, 2014).

The main objective of this study was to screen and characterize chromate resistant and reducing bacteria from the metal polluted environment, and to determine the chromium reduction potential of the isolated strain as a potential candidate for the chromate bioremediation.

2. Materials and methods

2.1. Sample collection and screening of chromium tolerant bacterial strain

Nine tannery effluent samples were collected from different locations of long term chromium contaminated sites of Sheikhpura and Qasoor, Lahore (Pakistan). At the time of sample collection, some physiochemical characteristics of effluent samples (color, pH, temperature) were noted. For cultivation of Cr⁶⁺ tolerant bacteria, serial dilutions of the wastewater samples were prepared, and plated onto modified Luria-Bertani (LB) agar [g/l; 10, trypton; 5, yeast extract; 5, NaCl; 15, agar] supplemented with 2 mM K₂Cr₂O₇, and incubated at 37 °C for 24–72 h. Bacterial isolates screening was done by their potential to survive on higher concentrations of hexavalent chromium (K₂Cr₂O₇). Only one Cr⁶⁺ resistant bacterial strain, A-HS1, was selected for further study on the basis of its resistance and pathogenicity (Fig. S1).

2.2. Biochemical and molecular characterization

The purified isolated strain A-HS1 was characterized by routine biochemical tests (Gram's staining, Catalase, Mannitol fermentation). Genomic DNA was isolated (Masneuf-Pomarade et al., 2007) and 16S rRNA gene was amplified by using the universal bacterial primers RS1 and RS3 through PCR (Rehman et al., 2007). PCR product was purified by Fermentas purification kit (#K0513) and Genetic analysis system model CEQ-800 (Beckman) Coulter Inc. Fullerton, CA, USA was used to analyze the sequence. The sequences were identified through BLAST analysis. Moreover, a dendrogram was constructed on the basis of homology using MEGA7 program.

2.3. Determination of optimum growth conditions

The temperature and pH optima were tested for the bacterial isolate. The strain was cultured in (LB) liquid medium (pH 7) at 20 °C, 30 °C, 37 °C, 45 °C and 55 °C. For the pH experiment, cultures (100 ml LB in 250 flasks) were adjusted to pH 5, 6, 7, 8, 9 and 10 and incubated at the optimum temperature. For growth pattern, bacterial isolate was grown in MS broth [g/l: FeSO₄.7H₂O 0.015 g, KH₂PO₄ 4.7 g, MgSO₄.7H₂O 1 g, CaCl₂.2H₂O 0.01 g, Na₂HPO₄ 0.12 g, NH₄NO₃ 4 g, MnSO₄.4H₂O 0.01 g, glucose 10 g and yeast extract 5 g (pH 7–7.2)] without metal (control) and with 2 mM K₂Cr₂O₇

(experimental) at 37 °C for 24 h. Optical density at 600 nm (OD_{600nm}) was employed as the function of bacterial growth.

2.4. Minimum inhibitory concentrations (MICs) of Cr⁶⁺ and other heavy metals

MIC of Cr⁶⁺ and other heavy metals was determined in modified M9 broth [g/l: Na₂HPO₄, 0.65 g; KH₂PO₄, 1.5 g; NH₄Cl, 0.5 g; NaCl, 0.25 g; MgSO₄.7H₂O, 0.12 g; Casamino acid, 10 g; Glucose, 5 g (pH 6.9)]. To check MIC for Cr⁶⁺, different concentrations of K₂Cr₂O₇ (0.05–30 mM) was supplemented to the 100 ml modified M9 medium and incubated at 37 °C in rotary shaker at 120 rpm for one week and cell growth was determined at OD₆₀₀. Similarly, to evaluate MICs for other heavy metals, different concentrations of CdCl₂, NiCl₂.6H₂O, ZnSO₄.7H₂O, CuSO₄.5H₂O and PbNO₃, ranging from 1 to 15 mM, were added separately to the modified M9 medium. All metal solutions were prepared in deionized water and filter sterilized.

2.5. Chromate reductase assay

2.5.1. Crude enzyme preparation

The bacterial isolate was cultivated in 100 ml MSM amended with 2 mM K₂Cr₂O₇ and incubated at 37 °C rotary shaker for 5 days. Cells were centrifuged at 6000 rpm for 10 min. The supernatant and pellet were separated, the pellet was washed thrice (phosphate buffer), and sonicated for 15 pulses with an interval of 60 s for 3 times. The sonicated pellet was centrifuged for 10 min at 14,000 rpm and the supernatant was used as intracellular crude enzyme. Ammonium sulphate was used to saturate the supernatant up to 60% and placed at –20 °C overnight. The mixture was centrifuged for 10 min, the upper 10 ml supernatant was discarded, and the pellet was used for extracellular enzyme. Chromate reductase activity, both extra and intra-cellular was assayed according to the method of Sarangi and Krishnan (2008).

2.5.2. Chromate reductase characteristics

The optimal pH value was scrutinized for maximum chromate reductase activity by using different buffers of pH values ranging from 4.0 to 9.0. The reaction mix contained 50 mM buffer of the specific pH with 20 μM Cr⁶⁺, 0.1 mM NADH and crude enzyme (0.1 ml), and incubated for 30 min. For optimum temperature, enzyme assay was done at various incubation temperatures i.e., 30, 40, 50, 70, 90 °C. The metal ions effect was assessed by adding 0.1 mM concentration of metal ion solutions i.e., NaCl, MnCl₂, MgCl₂, ZnCl₂, HgCl₂, NiCl₂, CuSO₄. The reaction mixture with no added metal served as control, and all the enzyme activities were evaluated by Sarangi and Krishnan (2008).

2.6. Quantification of antioxidant enzymes

To assay antioxidant enzymes, A-HS1 was cultivated in 100 ml MSM medium at 37 °C for 24 h. After 24 h growth, medium was supplemented with 2 mM Cr⁶⁺ and incubated again. After 48 h of incubation, cells were harvested at 14,000 rpm for 10 min, and pellet was weighed and resuspended in phosphate buffer. Pellet was sonicated and centrifuged, and the supernatant was used for the estimation of antioxidants.

Glutathione transferase (GST) activity was assayed according to Habig et al. (1974). Ascorbate peroxidase (APOX) activity was determined according to Israr et al. (2006). Peroxidase (POX) activity was determined by the method of Reuveni et al. (1992) and catalase activity was determined according to Beers and Sizer (1952). Superoxide dismutase (SOD) activity was measured indirectly as described by Ewing and Janero (1995).

2.7. Quantification of glutathione and cysteine levels in metal stress

To estimate levels of reduced glutathione (GSH), oxidized glutathione (GSSG), and other non-protein thiol in bacterial cell lysate under Cr^{6+} stress conditions, method of Shamim and Rehman (2013) was followed. Briefly, bacterial strain was cultivated for 24 h and then 2 mM $\text{K}_2\text{Cr}_2\text{O}_7$ was added in two flasks while the third one without metal stress served as control. The flasks were incubated again for 48 h. Cells were pelleted, washed thrice with phosphate buffer and pellet was weighed and re-suspended in 1 ml of 5% sulfosalicylic acid. Pellet was sonicated and centrifuged at 14,000 rpm at 4 °C for 10 min. The supernatant was divided into two equal portions, one was used to determine level of glutathione and the other was used for non-protein thiol estimation.

2.8. Metal uptake potential of the bacterial isolate

To assess metal uptake potential of the bacterium, A-HS1 was cultured in 100 ml LB broth with 2 mM Cr^{6+} stress. As a control in one flask medium was only supplemented with 2 mM Cr^{6+} but without bacterial culture. All the flasks were incubated in shaking incubator (120 rpm) at 37 °C. An aliquot of 5 ml was withdrawn from each flask after regular intervals (2, 4, 6 and 8 days of growth). Samples were spun down at 6000 rpm for 10 min and, both the pellets and supernatants were used for Cr^{6+} estimation by atomic absorption spectrophotometer (Zahoor and Rehman, 2009).

2.8.1. Tannery effluent Cr^{6+} bioremediation; a mini-pilot assay

A-HS1 potential to reduce Cr^{6+} in tannery effluents was determined. A set of three plastic containers was used; the first container carried only tannery wastewater (10 L) (control), second container carried 10 L distilled water with 1.5 L culture, while the third container carried 10 L wastewater with 1.5 L culture. Each container was amended with 2 mM Cr^{6+} and incubated at room temperature (25 ± 2 °C). Ten milliliter samples were taken after regular intervals (2, 4, 6, 8 days of incubation), and growth was centrifuged for 10 min at 4000 rpm. The samples supernatants were analyzed for Cr^{6+} estimation by DPC method. Change in Cr^{6+} concentration was determined by the standard curve made under the same experimental conditions using standards of Cr^{6+} solution.

2.9. Use of bacterial treated effluent for plant growth

Effect of bacterially treated tannery wastewater on plant growth was investigated. In this regard, small pots were prepared using autoclaved soil and six seeds of *Vigna radiata* (mung beans) were sown in each pot. The experimental pot was watered with wastewater treated with bacterial isolate A-HS1 while the other two controls were watered with tap water and original wastewater (untreated). The plants were cultivated for 10 days under 1:1 light and dark period. Any difference in plants growth watered with microbially treated wastewater and untreated wastewater, was observed

2.10. Bacterial protein isolation

The bacterium was cultivated in MSM broth with and without 2 mM Cr^{6+} . SDS PAGE was performed according to Laemmli (1970).

2.11. Fourier-transform infrared and energy dispersive X-ray analysis

Infrared spectra for A-HS1 cells were obtained using a Fourier Transform Infrared Spectrometer (Bruker, alpha). The method of Deokar et al. (2013) was employed to prepare bacterial samples. To ascertain the binding mechanism between the metal ions and microbial cells, it is imperative to identify the location of the chromium species (Cr^{6+} , Cr^{3+}) relative to the bacterial cells. Elemental analysis of hexavalent chromium reduction was determined by EDXA (energy dispersive X-ray analysis) to evaluate the compound distribution before and after the biosorption of heavy metal.

2.12. Statistical analysis

For each experiment, three independent measurements were taken and data shown are average values of means \pm standard deviation (SD). Significance testing between samples was calculated by performing paired Student's t-test. Control group was treated identically without exposure to any treatment.

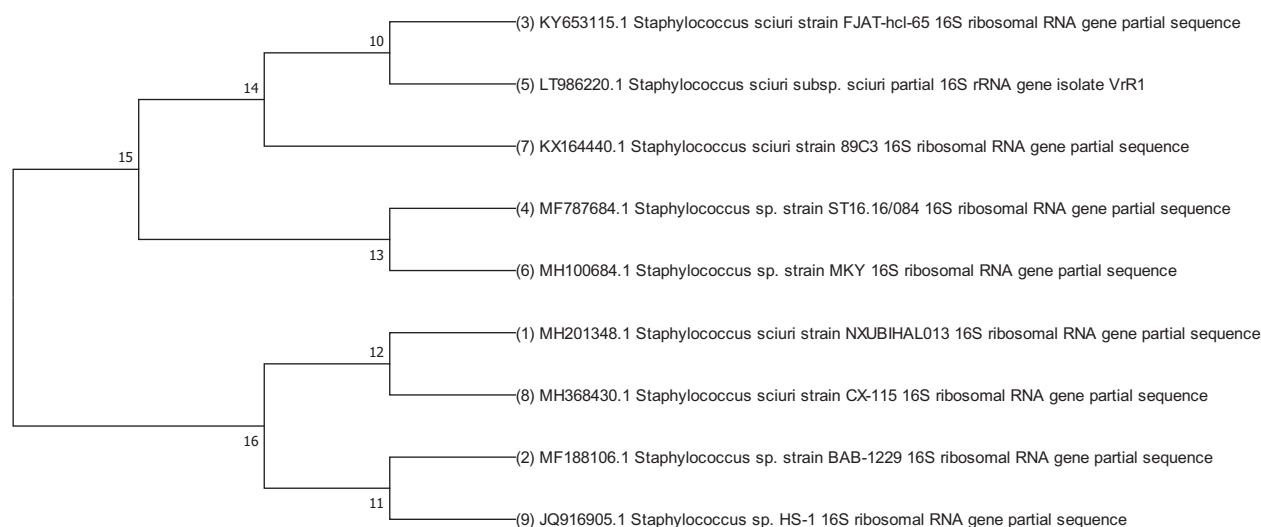


Fig. 1a. The evolutionary relationship of *S. sciuri* A-HS1 was inferred with already reported strains. The evolutionary history was deduced using the Neighbor-Joining method with 500 bootstrap test value in MEGA7 software.

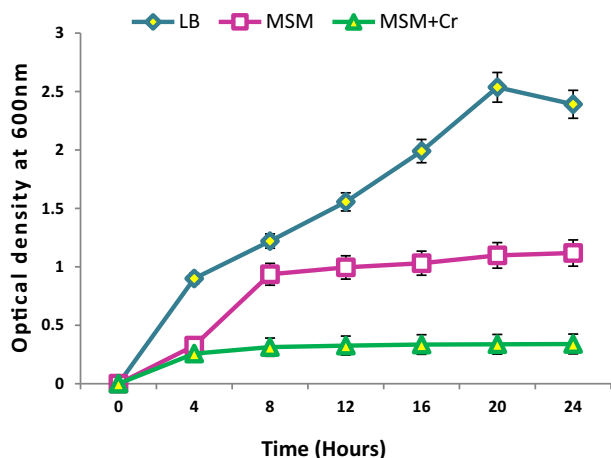


Fig. 1b. Growth curves of *Staphylococcus sciuri* A-HS1 in LB medium (control), mineral salt medium (MSM) and MSM supplemented with 2 mM $K_2Cr_2O_7$ (treated) incubated at 37 °C. Optical density was taken at 600 nm after regular time interval.

3. Results

3.1. Physiochemical characteristics

It was observed that the temperature of all the samples ranged from 22 to 34 °C, and pH range was 6.0–10.0. Samples were diluted and plated on LB agar plates amended with 2 mM Cr^{6+} . Concentration of Cr^{6+} was increased regularly, and one isolate who survived at the highest concentration of Cr^{6+} (25 mM) was selected for this study, labelled as A-HS1.

3.2. Identification of bacterial isolate

The organism is gram and catalase positive and characteristics of the isolate are given in Table S1. 16S rRNA gene ribotyping revealed 93% homology with *Staphylococcus sciuri* (Accession number JQ916905) already submitted to NCBI database. A dendrogram was constructed on the basis of similarity with other *S. sciuri* strains using MEGA7 program taking bootstrap value 500 (Fig. 1a).

3.3. Optimum growth conditions

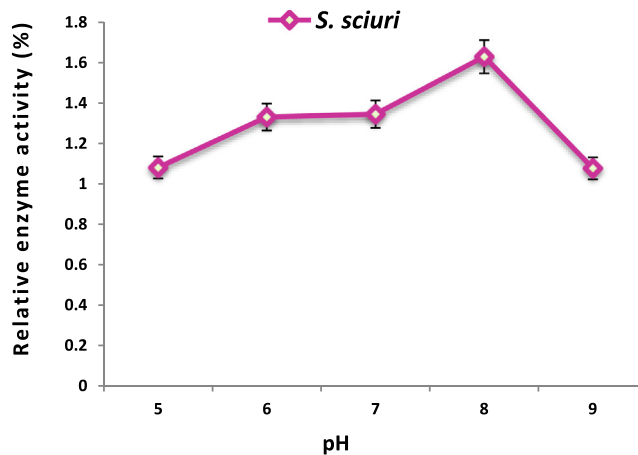
S. sciuri showed maximum growth at 37 °C and pH 7. Hexavalent chromium presence (2 mM Cr^{6+}) in the medium significantly decreased the bacterial growth (Fig. 1b).

3.4. Cross metal resistance

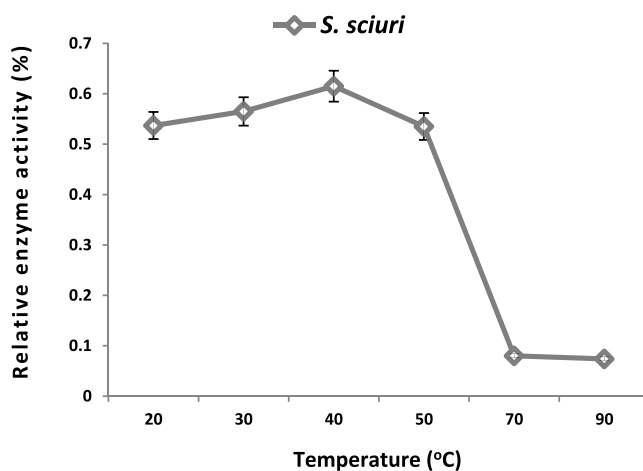
S. sciuri was capable to tolerate Cr^{6+} up to 25 mM and also showed resistance to other heavy metals, viz., 17 mM (Zn^{2+}), 18.5 mM (Pb^{2+}), 2.5 mM (Cu^{2+}), 3 mM (Cd^{2+}), 19 mM (As^{3+}) and 1.5 mM (Ni^{2+}). The order of resistance regarding heavy metals concentration was $Cr^{6+} > As^{3+} > Pb^{2+} > Zn^{2+} > Cd^{2+} > Cu^{2+} > Ni^{2+}$.

3.5. Characterization of chromate reductase

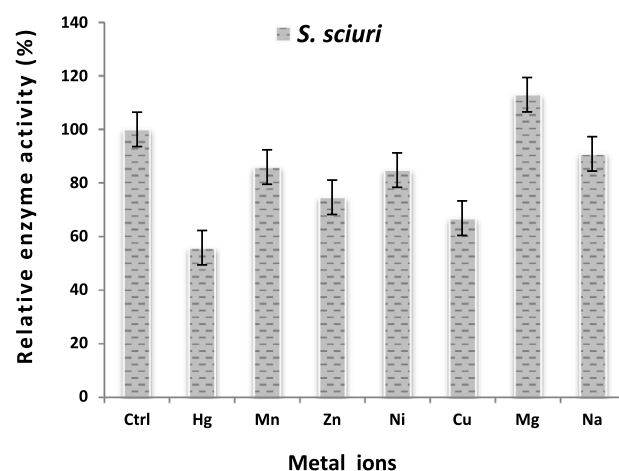
Characterization of chromate reductase of *S. sciuri* A-HS1 demonstrated optimum pH for the enzymatic activity was 8.0 (Fig. 2a) and optimum temperature was 40 °C, as the enzyme



(a)



(b)



(c)

Fig. 2. Effect of pH (a) and temperature (b) on enzyme activity (c) metal ions on the chromate reductase activity.

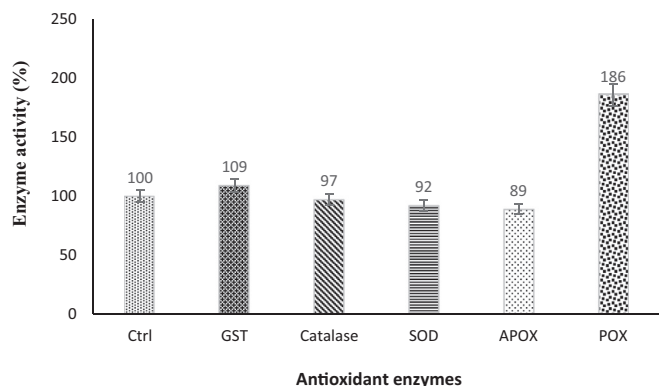


Fig. 3. Changes in antioxidant enzymes activity profile, exhibited by *S. sciuri* A-HS1 upon exposure of 2 mM Cr⁶⁺.

was inhibited significantly at 70 °C (87% of inhibition) (Fig. 2b). Effect of different metal cations on the enzyme activity (Fig. 2c) reveal that all the metal ions tested inhibit the Cr⁶⁺ reductase activity except for Mg²⁺ (13%).

3.6. Quantification of antioxidant enzymes

Estimation of the antioxidant enzymes in the presence of Cr stress unveils very interesting results. Cr⁶⁺ did not stimulate activities of APOX, SOD and CAT in significant amount, however, a decrease in activities of these enzymes was observed i.e., APOX (11%), SOD (8%), and CAT (3%), respective to the normal growth conditions. The Cr toxicity stimulated *S. sciuri* A-HS1 to express a relatively higher level of GST. The only antioxidant which expressed itself in significantly higher level was POX (86%) under Cr⁶⁺ stress condition (Fig. 3).

3.7. Quantification of GSH and non-protein thiols

An alternative level of GSH and GSSG was estimated in the presence of Cr⁶⁺ in *S. sciuri* A-HS1 (Table 1). When the bacterium was supplemented with 2 mM Cr⁶⁺ stress, an increase level of 230.8% in GSH was determined as compared to the control. Moreover, a significant increased level of non-protein thiols was also recorded in the presence of chromium (432.7%).

3.8. Chromium processing ability of bacterial isolate

3.8.1. Biosorption of Cr⁶⁺

Biosorption ability of *S. sciuri* A-HS1 was evaluated by culturing it in LB broth containing 2 mM Cr⁶⁺ (Fig. 4a). The biosorption efficiency (*q*) of *S. sciuri* A-HS1 was as 42, 73, 85 and 31 mM Cr⁶⁺/g after 2, 4, 6 and 8 days of incubation. The amount of Cr⁶⁺ up taken by bacterial cells after 2, 4, 6 and 8 days was 14, 16, 14 and 6 mM/g, respectively. While 22, 47, 63 and 16 mM/g of Cr⁶⁺ were estimated to be adsorbed on the surface of bacterium after the incubation of same time period.

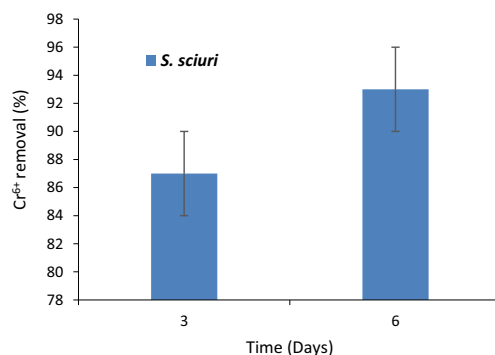
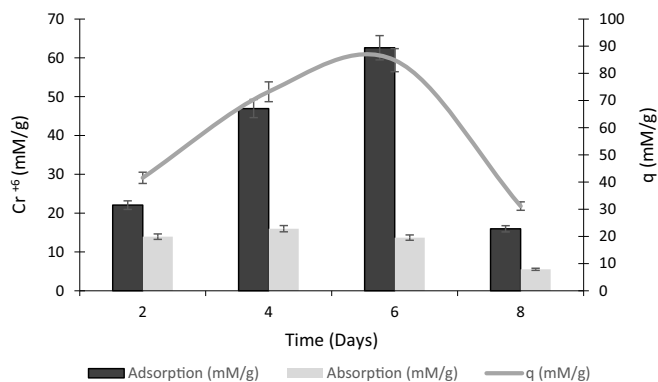


Fig. 4. (a) Biosorption of Cr⁶⁺ by *S. sciuri* A-HS1 at lab scale incubated for 8 days. (b) Cr⁶⁺ reduction by *S. sciuri* A-HS1 from tannery effluent after 3 and 6 days of incubation at room temperature.



Fig. 5. Growth of *Vigna radiata* (mung beans) in microbial treated wastewater (with *S. sciuri* A-HS1), tap water and untreated wastewater after 10 days of incubation under 1:1 light and dark period.

Table 1

Levels of reduced (GSH) and oxidized glutathione (GSSG), total glutathione, reduced and oxidized glutathione ratio, and nonprotein thiols in *S. sciuri* A-HS1 exposed to Cr⁶⁺ at 2 mM.

CrConc. (mM)	GSH(mMg ⁻¹ FW)	GSSG (mMg ⁻¹ FW)	GSH + GSSG (mMg ⁻¹ FW)	GSH/GSSG ratio	% increase in GSH	Non-protein thiols	% increase in Non-protein thiols
0.0	17.526	7.573	25.099	2.314	230.8	18.392	432.7
2.0	19.834	11.540	31.374	1.719		22.719	

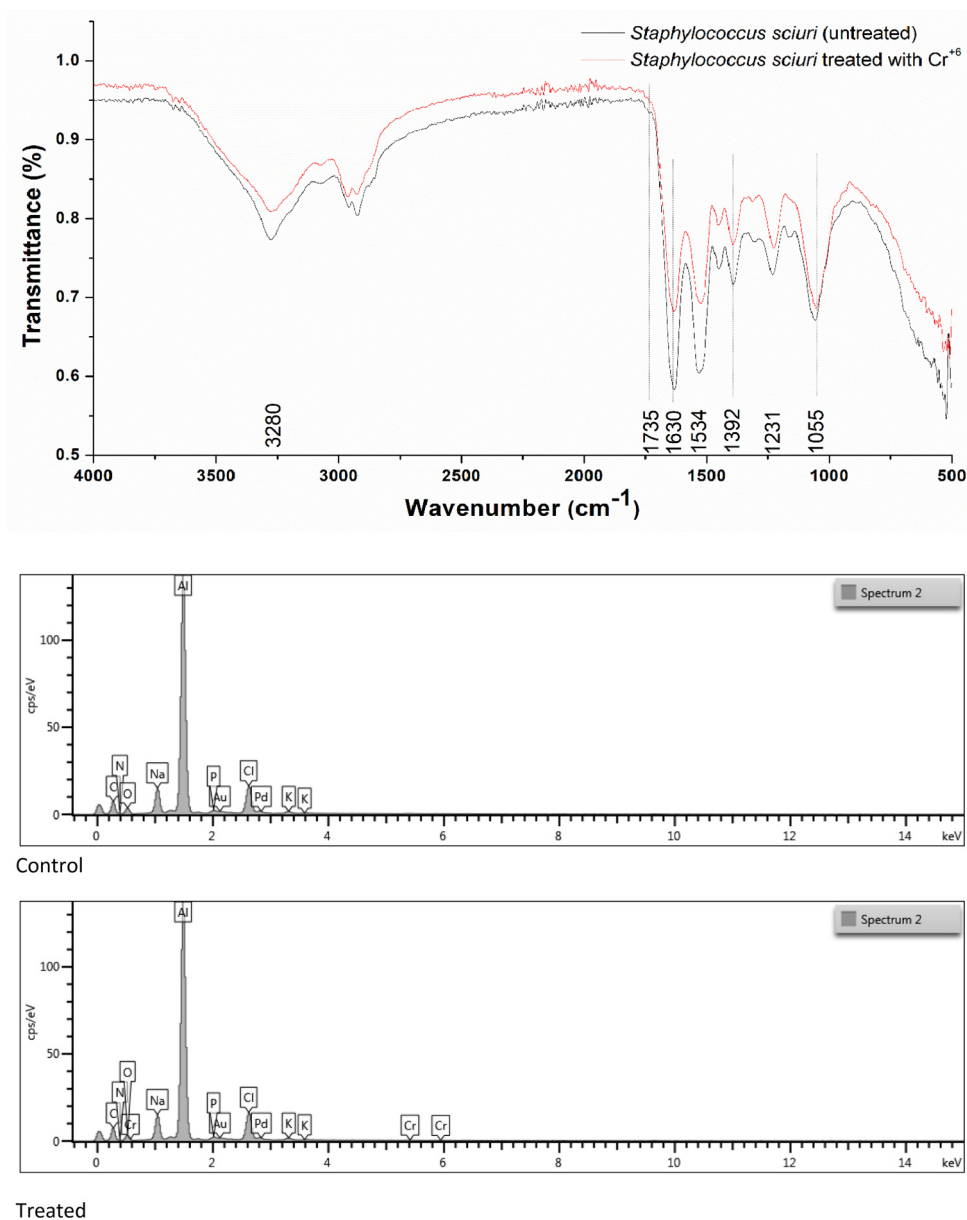


Fig. 6. (a) FTIR spectra of *S. sciuri* A-HS1 in the presence and absence (control) of hexavalent chromium stress (2 mM Cr^{6+}). (b) Energy dispersive X-ray spectroscopy through SEM of *S. sciuri* A-HS1 (A) control and (B) Cr^{6+} -treated. (c) Proposed Cr^{6+} resistance and reduction mechanism in gram positive bacterium, *S. sciuri* 43C. (a) Cr^{6+} reduces into Cr^{3+} extracellularly by chromate reductase. (b) Cr^{6+} enters into cell cytoplasm and changes into Cr^{3+} by intracellular chromate reductase. Some portion of Cr^{6+} r changes into its intermediate species. (c) Cr^{5+} and Cr^{4+} are involved in the generation of ROS. The oxidative stress resulted by the formation of ROS is combated by two ways; (d) antioxidant enzymes and (e) antioxidant molecules. After certain level of accumulation Cr^{6+} expel out through efflux system.

3.8.2. Pilot scale Cr^{6+} bioremediation

S. sciuri A-HS1 ability to reduce Cr^{6+} was checked at pilot scale, where 10 L of tannery wastewater was used and the efficiency of the organism was determined by DPC method. Assay results revealed that at pilot scale *S. sciuri* A-HS1 was able to remove 93% Cr^{6+} from tannery effluent after 6 days of incubation when Cr^{6+} concentration was maintained at 2 mM in tannery effluent (Fig. 4b).

3.9. Phytotoxicity study

In the present study phytotoxicity results showed good germination rate of *Vigna radiata* in the bacterial processed tannery wastewater and in tap water. The noxious tannery wastewater in the presence of bacterial strain has become nontoxic due to toxic Cr^{6+} reduction into less toxic Cr^{3+} (Fig. 5).

3.10. Protein analysis

Changes in total protein profiles of *S. sciuri* A-HS1 in the presence and absence of Cr^{6+} stress was checked by polyacrylamide gel electrophoresis. SDS-PAGE analysis revealed the over-expression of some proteins in the presence of Cr^{6+} . A protein band of about 65 kDa was observed in control culture sample but was absent in the culture which was challenged with 2 mM Cr^{6+} (Fig. S2).

3.11. FTIR and EDX analysis

FTIR spectrum of the control (without chromium) and treated (with chromium) bacterial strain *S. sciuri* A-HS1 is shown in

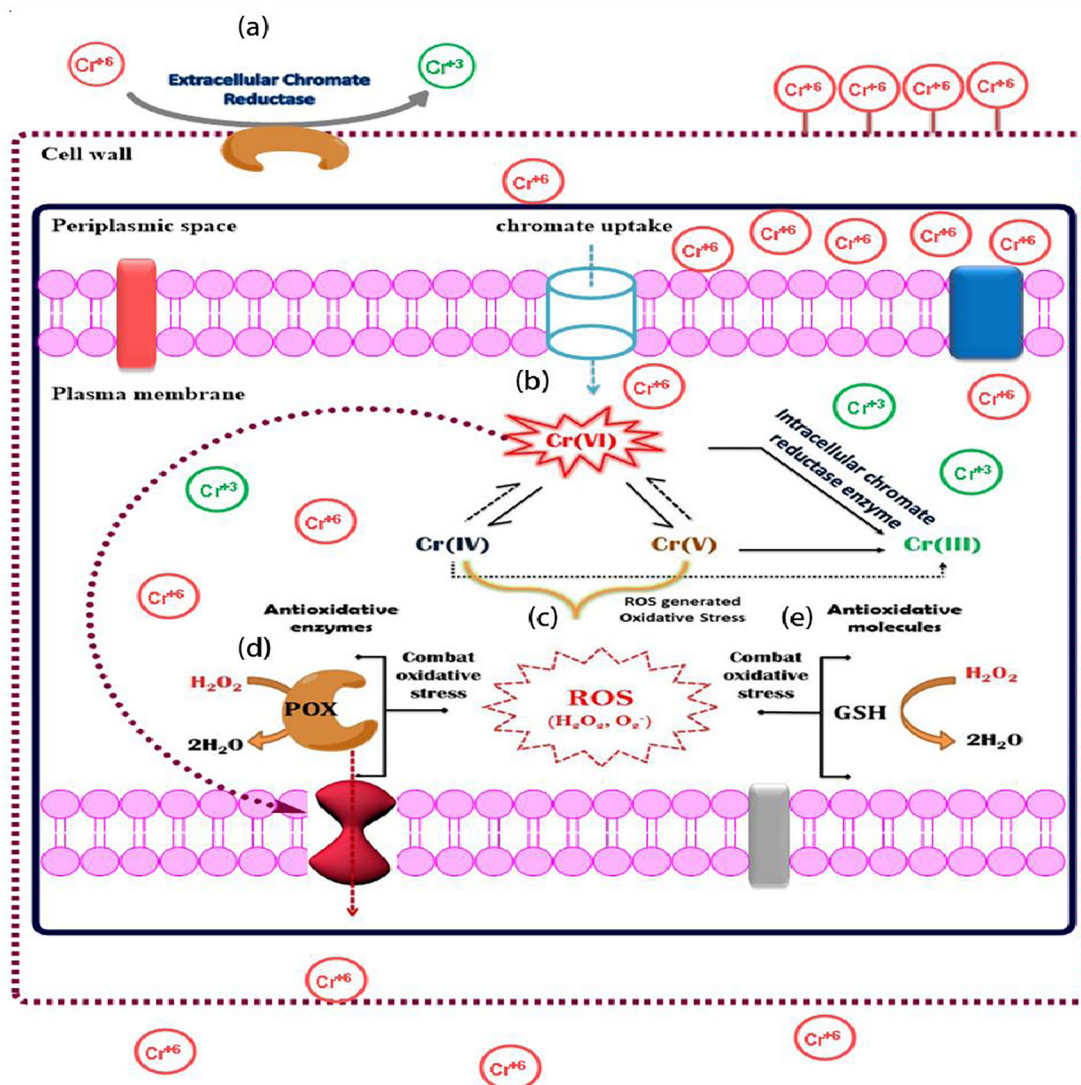


Fig. 6 (continued)

Fig. 6a. When grown in LB broth medium without Cr^{6+} , the infrared absorption peaks of *S. sciuri* A-HS1 display characteristic absorption peaks of amino, carboxyl, hydroxyl, and sulphonate groups representing the presence of moieties on the cell surface. While a shift in characteristic absorption peaks was observed under the stress of 2 mM Cr^{6+} . Under the stress of Cr^{6+} , *S. sciuri* A-HS1 displayed changes in the region of $1800\text{--}1000\text{ cm}^{-1}$ and 3280 cm^{-1} . EDX analysis also confirmed the accumulation of Cr^{6+} in the cell cytoplasm (Fig. 6b).

4. Discussion

Several investigations have been reported on chromate resistant and reducing bacteria, isolated from metal contaminated as well as non-contaminated environments (Zahoor and Rehman, 2009; Mistry et al., 2010; Kouadjo and Zeze, 2011; Sagar et al., 2012) (Table S2) under aerobic and anaerobic conditions (Wani et al., 2007; Tripathi and Garg, 2014; Iyengar and Usha, 2016). In this study, a bacterial strain, *S. sciuri* A-HS1 was isolated that could resist Cr^{6+} stress up to 25 mM. Chromate resistant *Staphylococci* species have also been reported previously in Cr^{6+} reduction (Mangaiyarkarasi and Geetharamani, 2014; Iyengar and Usha, 2016; Dutta et al., 2017).

Bioaccumulation comprises two stages. In the first step, initial uptake of metal take place through direct physical contact, that does not require energy, and this passive accumulation of metal at cell surface is thought to be mediated by adsorption, chelation, ion-exchange, complexation, and microprecipitation (Bharagava and Mishra, 2017). In the second stage, the initial rapid physical adsorption transforms into slow phase of chemical bondage of Cr^{6+} to high affinity sites. After bioaccumulation, Cr^{6+} could also get reduced to Cr^{3+} by serving as terminal electron acceptor before binding chemically to the cell wall (Srinath et al., 2001; Cheunga and Gu, 2007). It has been reported that there is a chemical analogy between sulphate and chromate oxyanions, therefore, active transportation of chromate ions across biological membranes could be mediated through sulphate channels (Cervantes and Campos-Gracia, 2007).

Once get inside the cell, Cr^{6+} rapidly takes part in a number of processes causing high toxicity in the cytoplasm (Bharagava and Mishra, 2017). Reduction of Cr^{6+} into Cr^{3+} occurs through enzymatic as well as non-enzymatic reactions under aerobic as well as anaerobic conditions via electron transport system containing cytochromes. Aerobically, reduction of Cr^{6+} occur through the production of toxic short lived intermediates such as of Cr^{5+} and Cr^{4+} (Bharagava and Mishra, 2017). However, it is not known that the

conversion of Cr⁵⁺ to Cr⁴⁺ and Cr⁴⁺ to Cr³⁺ is either enzyme mediated or spontaneous. In Cr⁶⁺ reduction, electron donors (NADH, NADPH) and electron from the endogenous reserve are also involved (Varadhan and Mohan, 2017). Tripathi et al. (2011) reported that *B. cereus* was able to bioremediate 74.5% Cr⁶⁺ after 48 h of incubation. In the present investigation, pilot scale study of Cr⁶⁺ bioremediation also replicated the results as the maximum removal of Cr⁶⁺ was noted after 6 days of incubation in case of real tannery effluent (93% Cr⁶⁺ removal).

To combat the heavy metal and oxidative stress in the cell, ROS generation imbalances the reducing environment within the cell, several proteins are produced by the defensive system to resist chromate stress (Ramirez-Diaz et al., 2008; Thatoi et al., 2014). However, ROS generated oxidative stress is largely combated by the antioxidant enzymes, that scavenge ROS by converting them into non harmful radicals, like superoxide dismutase (SOD), glutathione transferase, catalase, etc. (Ackerley et al., 2004; Hussein and Joo, 2013). Our results revealed that among antioxidants, the induction of APOX (11%), SOD (8%), and CAT (3%) is less than POX (86%) enzyme under Cr⁶⁺ stress. Peroxidases, widely known as stress enzymes, produced under various stress conditions such as drought-stress (Zhang and Kirkham, 1994), water stress (Zhang and Kirkham, 1994), salinity (Mittal and Dubey, 1991), gamma-radiation (Hussein and Joo, 2013) and under the toxic level of heavy metals such as Cu, Al, Cd, Zn (Chaoui et al., 1997; Hussein and Joo, 2013). Our results are in agreement with Chen et al. (2000) and Lee and Shin (2003) who have reported a slight increase in catalase activity after Cd stress. Our results are also consistent with Shamim and Rehman (2013), who revealed that the stress of Cd could not able to induce higher production of SOD, APOX, and GR, however, an increase in glutathione was estimated (Fig. 6c).

Energy Dispersive X-ray analysis confirmed the presence and accumulation of Cr⁶⁺ in the bacterial cell. FTIR spectroscopy was performed to identify the functional groups present on the bacterial cell surface. The peaks appearing between 3450–3200 cm⁻¹ are characteristic of the hydroxyl groups, and –NH groups moieties on the cell surface (Park et al., 2005; Mungasavalli et al., 2007). The intense bands within 1100–1000 cm⁻¹ represent C–O bond, attributed to the characteristic peak for polysaccharides (Das and Guha, 2007). The representative band region at 1100–1030 characterizes the presence of orthophosphate (PO₄³⁻). However, the same wavelength in the absorption spectrum i.e., 1350–1000 cm⁻¹, is also representing C–N stretching (Mungasavalli et al., 2007). Under the stress of Cr⁶⁺, *S. sciuri* A-HS1 displayed changes in the region of 1800–1000 cm⁻¹ and 3280 cm⁻¹. The shift in characteristic absorption peaks suggested that hexavalent chromium ions were sequestered by certain functional groups present on the cell surface (Bueno et al., 2008).

Utilization of bacterially treated wastewater for plantation purposes revealed that the seeds watered with the treated tannery wastewater showed a marked increase in plant growth as compared to the one watered with the original wastewater. The plants, watered with untreated wastewater, show delayed and hindered growth. Thus, bacterially treated wastewater could be safely used for the watering of plants in real time scenario.

5. Conclusions

S. sciuri showed high resistance against multiple heavy metals and was also capable to remove nearly 93% Cr⁶⁺ from the tannery wastewater over a period of 6 days. Moreover, bacterially treated tannery wastewater, which was found safe for the plantation, could be used at-least for crops irrigation. In coming years, it can become an attractive environmental tool for green chemistry after exploring its molecular biology.

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Conflicts of interest

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jksus.2018.07.016>.

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