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Comparative behavior of two gram positive Cr^{6+} resistant bacterial strains *Bacillus aerius* S1 and *Brevibacterium iodinum* S2 under hexavalent chromium stress

Amina Elahi, Abdul Rehman*

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

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ABSTRACT

Bacillus aerius S1 and *Brevibacterium iodinum* S2 showed maximum growth at 37 °C and pH 8. *B. aerius* and *B. iodinum* could resist Cr^{6+} upto 30 and 35 mM and biosorption proficiency (q) of *B. aerius* S1 was 19, 27, 52 and 34 mM/g while for *B. iodinum* S2, it was 39, 50, 23 and 16 mM/g mM/g after 2, 4, 6 and 8 days of incubation. A pronounced rise in antioxidant enzymes activities was determined in *B. aerius* S1 i.e. POX (963%), CAT (717%), APOX (699%), SOD (683%), and GST (792%). However, in *B. iodinum* S2, relatively a minor increase was estimated. A significant GSH increase was determined in *B. aerius* S1 (364%) and *B. iodinum* S2 (663%) cultures under 2 mM Cr^{6+} stress. Pilot scale study demonstrated that both strains could reduce Cr^{6+} into Cr^{3+} within 6 days from the original tannery effluent with efficiency of 99%.

1. Introduction

Industrial revolution and technological advancement where raised the standards of living, is a major cause of the environmental pollution. This is due to the fact that industries discharge tons of hazardous wastes containing heavy metals (chromium, cadmium, and lead), metalloids, and organic pollutants at elevated concentrations that have wreaked severe damage to the environment [1,2]. Continuous release and non-degradability of metalloids and heavy metals make them persistent in the biosphere thus posing serious global health issues. The alarming concentration of heavy metals in the environment and its subsequent detrimental consequences to all life forms underline the need of immediately applying effective techniques to cut down their concentration to acceptable limit [3,4].

Chromium has been extensively used in industrial operations which include metal finishing industry, petroleum refinery, electrolating, leather tanning, iron and stainless steel industries, water cooling and wood preservation and pulp processing industries [5], paint and pigment manufacturing, textile and fertilizer industries [6]. These industries produce huge amounts of solid and liquid waste materials harboring Cr^{6+} compounds which easily dissolved in water causing toxicity and carcinogenicity in mammals [7]. Environment Protection Agency has listed Cr^{6+} compounds such as chromate and dichromate as priority pollutants in USA [8]. On the other hand, Cr^{3+}, is comparatively far more less toxic and readily get precipitated at higher pH than 5.5, formatting insoluble oxides and hydroxides that precipitates rapidly in soil and water systems [7,9].

A potential detoxification process comprises conversion of Cr^{6+} into Cr^{3+} that could be accomplished via physiochemical or biological methods. To detoxify Cr^{6+} contaminated sites, conventional technologies could be applied such as land filling, soil washing, flushing, excavation, and physico-chemical extraction, but, these methods utilize chemical reagents and are quite expensive and cumbersome [9]. Therefore, it is need of the day to establish an innovative, economical and eco-friendly method to remove toxic heavy metal from the wastewater. Thus, bioremediation is the approach of choice which utilize the indigenous microbiota to clean-up heavy metals from the contaminated environment thus the reestablishing the polluted area without addition of chemical reagents [10]. Various microbes, for instance, bacteria, fungi, algae and protozoa are habitually residing in water mixed with industrial effluents, and these residing microbes have developed strategies to combat the heavy metal toxicity via processes like metal uptake, methylation, adsorption, oxidation and reduction [11].

The objective of the present study was to isolate, characterize, and determine the Cr-removal potential of indigenous micro-flora from the industrial wastewater. Two Cr^{6+} resistant gram positive strains *Bacillus aerius* S1and *Brevibacterium iodinum* S2 from tannery effluent were isolated and characterized. Besides, the behavior of antioxidant enzymes activities were observed under Cr^{6+} stress, which scavenge the reactive oxygen species produced under

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

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heavy metal stress. Furthermore, Cr^{6+} adsorption, and its subsequent accumulation in the bacterial cell were observed through FTIR spectroscopy, and SEM-EDX analysis, respectively. The bioremediation potential of these bacterial strains was ascertained on the basis of metal-resistance and reducing Cr^{6+} into Cr^{3+}.

2. Material and methods

2.1. Isolation and characterization of Cr^{6+} resistant bacterial isolate

Bacterial isolates were obtained indigenously from the tannery effluent from the industrial sites of Sheikhupura, and Qasoor, Lahore (Pakistan). Tannery effluent samples were diluted before plating onto the Luria-Bertani (LB) agar amended with 1 mM Cr^{6+} stress in the form of $K_2Cr_2O_7$ and incubated for 24–72 h at 37 °C. Screening of the bacteria was done on the basis of their ability to resist and reduce higher Cr^{6+} concentrations.

Molecular characterization of the isolated pure strains S1 and S2 were done. Methods of Masneuf-Pomarède et al. [12] was utilized to isolate genomic DNA of the bacterial isolates S1 and S2 and 16S rRNA gene was amplified using primers 8 F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [13]. The amplified PCR products were cleaned with the Fermentas purification kit (#K0513) and sent for sequencing from Macrogen, Korea. To align the nucleotide sequences, basic local alignment search tool (BLAST) analysis was used.

2.2. Determination of optimum growth conditions

Optimal cultivation conditions of S1 and S2 were ascertained. Optimal growth temperature of S1 and S2 were determined by growing bacterial strains in 100 ml LB broth contained in 250 ml flask and incubated at four different temperatures i.e. 20 °C, 30 °C, 37 °C and 50 °C, for 24 h. cell growth of the bacterial cultures were obtained by taking OD at 600 nm. For optimal pH of S1 and S2, different pH values of the LB broth (5, 6, 7, 8, 9, and 10) were set, and inoculated with the log phase bacterial isolates. These flasks were placed in shaking incubator at 37 °C for 24 h and cell densities were determined at 600 nm.

Growth profiles of the bacterial isolates S1 and S2 in the absence and presence of Cr^{6+} was studied. Bacterial strains were cultivated in mineral salt medium (MSM) broth [g/L: $FeSO_4.7H_2O$ 0.015 g, KH_2PO_4 4.7 g, $MgSO_4.7H_2O$ 1 g, $CaCl_2.2H_2O$ 0.01 g, Na_2HPO_4 0.12 g, NH_4NO_3 4 g, $MnSO_4.4H_2O$ 0.01 g, glucose 10 g and yeast extract 5 g (pH 7–7.2)] without metal (control), and MSM broth containing 2 mM $K_2Cr_2O_7$ (treated). The cell density was obtained at $O.D_{600\ nm}$ after regular intervals until 24 h of incubation.

2.3. Determination of MICs of Cr^{6+} and other heavy metals

MICs of heavy metals against S1 and S2 were determined. For this, different concentrations of metal salts including $K_2Cr_2O_7$ (for Cr^{6+}), $CdCl_2$, $CuSO_4.5H_2O$, $NiCl_2.6H_20$, $PbNO_3$ and $ZnSO_4.7H_2O$ were separately added to 100 ml modified M9 broth medium [g/L: Na_2HPO_4, 0.65 g ; KH_2PO_4, 1.5 g ; NH_4Cl, 0.5 g ; NaCl, 0.25 g ; $MgSO_4.7H_2O$, 0.12 g; Casamino acid, 10 g; Glucose, 5 g (pH 6.9)]. All the flasks were inoculated with log phase culture of S1 and S2, separately and placed in shaking incubator at 37 °C at 150 rpm for 7 days. Optical density was taken, as cell growth of the bacterial isolates, at $OD_{600\ nm}$. Lowest metal concentration that is able to inhibit bacterial growth was considered as MIC.

2.4. Quantification of antioxidant enzymes and glutathione contents

Behavior of antioxidant enzymes of bacterial strains S1 and S2 was studied under Cr^{6+} stress. For this, bacterial strains were grown in 100 ml MSM medium in 250 ml flasks and placed in shaking incubator at 37 °C. After 24 h of incubation, 2 mM Cr^{6+} stress was added in the media and flasks were incubated again for another 24 h. Cultures were centrifuged at 14,000 rpm for 10 min, and pellets were weighed and dissolved in phosphate buffer and sonicated. The aliquots obtained after centrifugation of sonicated pellets were used for assaying antioxidant enzymes. Methods of Habig et al. [14] was used to evaluate glutathione transferase (GST) activity. Peroxidase (POX) enzyme was assayed according to Reuveni et al. [15] with minor modifications. Catalase, ascorbate peroxidase (APOX) and superoxide dismutase (SOD) activities were determined by the methods of Beers and Sizer [16], Israr et al. [17], Nakano and Asada [18], and Ewing and Janero [19], respectively.

Any alteration in the induction of glutathione and other non-protein thiols under chromium stress was determined according to Khan et al. [20]. For each strain, three flasks of medium were prepared and inoculated with bacterial culture. After 24 h of incubation, two culture flasks were supplemented with stress of 2 mM $K_2Cr_2O_7$ stress, and third flask with no metal act as control. All the flasks were incubated again for another 48 h. After incubation, cultures were centrifuged, washed with 1 mM phosphate buffer, weighed and re-suspended in 1 ml of 5% sulfosalicyclic acid. The cell pellets were subjected to sonication and centrifuged at 14,000 rpm for 10 min at 4 °C and aliquot was separated into two equal parts. One part was used to assess glutathione level and other part was used to estimate non-protein thiol levels. Levels of reduced glutathione (GSH), oxidized glutathione (GSSG) and non-protein thiols were quantified by Khan et al. [20].

2.5. Metal processing potential of the Bacterial isolates S1 and S2

Metal processing ability of the bacterial strains S1 and S2 were evaluated by measuring changes in the quantity of Cr^{6+} in the culture medium by atomic absorption spectrophotometer according to Rehman et al. [21]. For each strain, two flasks were used for bacterial growth under 2 mM Cr^{6+} stress while the flask with 2 mM Cr^{6+} containing no organism served as control. Flasks were placed in shaking incubator at 37 °C and 120 rpm. After regular time interval i.e. 2, 4, 6, and 8 days, 5 ml aliquot were taken from each flask and cell culture was centrifuged at 6000 rpm for 10 min. Both the pellets and supernatants were used for Cr^{6+} estimation.

The pellets were washed with autoclaved distilled water, weighed and separated into two equal portions. To collect the adsorbed Cr fraction on the cell surface as a soluble fraction, one ration was washed with 0.5 M EDTA three times while the other ration was acid digested to gather the absorbed Cr. Acid digestion of pellet was done by re-suspending it in 1 ml autoclaved distilled water along with 1 ml of 0.2 N HNO_3 (1:1) and placed on hot plate for 30 min till the suspension turned yellow. This aliquot was used to calculate intracellular Cr concentration. Standard curve of chromium was employed to calculate concentration of metal.

2.6. Chromate reduction in tannery effluent

The Cr^{6+} reduction potential of S1 and S2 was determined in tannery effluent. Three plastic containers were used; the first container carried the control 1 (10 l original tannery wastewater) while the second container carried control 2 (10 l distilled water, inoculated with 1.5 l culture), and the third container was filled with tannery wastewater (10 L) with culture. All containers were given 2 mM Cr^{6+} stress and incubated at room temperature (25 ± 2 °C). Samples (10 ml) were withdrawn after regular time of incubation (2, 4, 6, 8 days). Cells were centrifuged at 4000 rpm for 10 min and residual Cr^{6+} concentration was determined from supernatants via Diphenylcarbazide method. Alterations in concentration of Cr^{6+} after bacterial treatment was calculated from the

calibration curve established under the same experimental conditions using a standards of Cr^{6+} solution.

2.7. FTIR, SEM and EDX analysis

FTIR Spectroscopy (Bruker, alpha) was employed to obtain Infrared spectra for S1 and S2 under Cr^{6+} stress. Specimens were treated as mentioned by Deokar et al. [22]. To determine the mechanism of metal-microbe interaction, it is essential to locate the presence of chromium ions (Cr^{6+}, Cr^{+3}) in the bacterial cells. To confirm the intracellular accumulation of chromium SEM-EDX analysis was done.

For scanning electron microscopy, samples were prepared according to Khan et al. [23]. In short, bacterial culture with and without 2 mM Cr was prepared and a drop of suspension was mounted onto the aluminum stub and treated as described by Khan et al. [23]. With sputter coater, samples were covered with gold film (Denton, Desk V HP) and examined through scanning electron microscope (Nova NanoSEM 450) equipped with Oxford energy dispersive X-ray (EDX) microanalysis system.

2.8. Statistical analysis

All the experiments run in triplicate and observations were made. Each experiment was done in at least three separate flasks. Each time three readings were taken, their mean, and standard error of the mean were calculated.

3. Results

3.1. Screening of chromate resistant bacterial strains

Serial dilutions of the effluent samples were plated on to the LB agar plates supplemented with 2 mM Cr^{6+}. The stress of Cr^{6+} in the medium was increased gradually, and the isolates resisted high Cr^{6+} concentration as well as have the ability to reduce Cr^{6+} were selected. Two selected strains, labeled as S1 and S2 were capable of resisting 30 and 35 mM Cr^{6+}, respectively. Both isolates S1 and S2 are gram positive and 16S ribotyping showed 100% homology of S1 with *Bacillus aerius* and S2 with *Brevibacterium iodinum* (Accession number KX941840 and KX941841 respectively) already submitted to NCBI database.

3.2. Optimum growth conditions

B. aerius S1 and *B. iodinum* S2 optimum growth temperature was determined as 37 °C and pH 8 for *B. aerius* S1 while pH 8 for *B. iodinum* S2. Growth of both strains was substantially declined in presence of Cr^{6+} (Fig. 1).

3.3. Cross metal resistance

B. aerius S1 and *B. iodinum* S2 could resist Cr^{6+} up to 30 and 35 mM, respectively. Both strains are capable of tolerating other heavy metals as well, however, the pattern of resistance vary. For *B. aerius* S1, viz. 23 mM (Pb^{2+}), 17 mM (Zn^{2+}), 2 mM (Cu^{2+}), 5 mM (Cd^{2+}), 21 mM (As^{3+}) and 3 mM (Ni^{2+}). Resistance order according to metal ions concentration was $Cr^{6+} > Pb^{2+} > As^{3+} > Zn^{2+} > Cd^{2+} > Ni^{2+} > Cu^{2+}$. For *B. iodinum* S2, viz., 9 mM (Pb^{2+}), 17 mM (Zn^{2+}), 3 mM (Cu^{2+}), 1 mM (Cd^{2+}), 2 mM (As^{3+}) and 3 mM (Ni^{2+}). Resistance order according to metal ions concentration was $Cr^{6+} > Zn^{2+} > Pb^{2+} > Ni^{2+} / Cu^{2+} > As^{3+} > Cd^{2+}$.

3.4. Quantification of antioxidant enzymes and glutathione

Under Cr^{6+} stress the antioxidant enzyme profiling of the two bacterial isolates, *B. aerius* S1 and *B. iodinum* S2, showed interesting results. In *B. aerius* S1, a pronounced rise in activities of all the antioxidant enzymes was observed i.e. POX (963%), CAT (717%), APOX (699%), SOD (683%), and GST (792%). However, in *B. iodinum* S2, relatively only a minor increase in enzyme activities of GST (17%), CAT(27%), SOD (16%), and POX (34%), moreover, 1% decrease in APOX was noticed (Fig. 2).

Cr^{6+} stress also stimulates GSH and GSSG levels in both *B. aerius* S1 and *B. iodinum* S2, (Table 1). In *B. aerius* S1 and *B. iodinum* S2, under stress of 2 mM Cr^{6+}, 364% and 663% increase in GSH was determined as compared to the control, respectively. Also, rise in non-protein thiols was observed in *B. aerius* S1 (275%) and *B. iodinum* S2 (177%) (Table 1).

3.5. Chromium processing ability of bacterial isolates

3.5.1. Biosorption of Cr^{6+}

Biosorption potential of *B. aerius* S1 and *B. iodinum* S2 was assessed by cultivating them in LB broth supplemented with 2 mM Cr^{6+} (Fig. 3). Biosorption proficiency (q) of *B. aerius* S1 was determined after 2, 4, 6 and 8 days which was 19, 27, 52 and 34 mM/g, respectively. While for *B. iodinum* S2, the q value after 2, 4, 6 and 8 days was estimated as 39, 50, 23 and 16 mM/g, respectively (Fig. 3).

3.5.2. Pilot study of Cr^{6+} bioremediation

Reduction potential of *B. aerius* S1 and *B. iodinum* S2 was also determined at pilot scale, where the efficiency of the isolates were

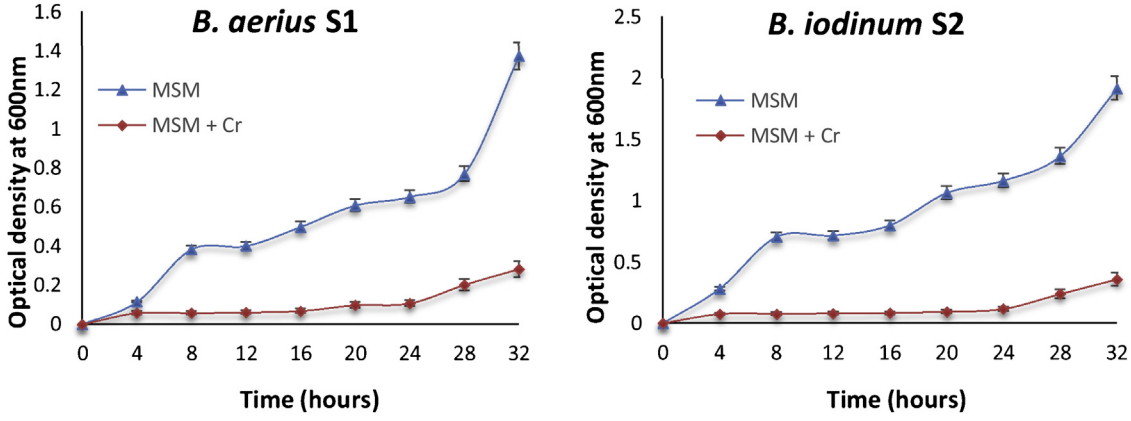

Fig. 1. Growth curves of *B. aerius* S1 and *B. iodinum* S2; in mineral salt medium (control) and MSM supplemented with 2 mM $K_2Cr_2O_7$ (treated) incubated at 37 °C. Optical density was taken at 600 nm after regular time interval.

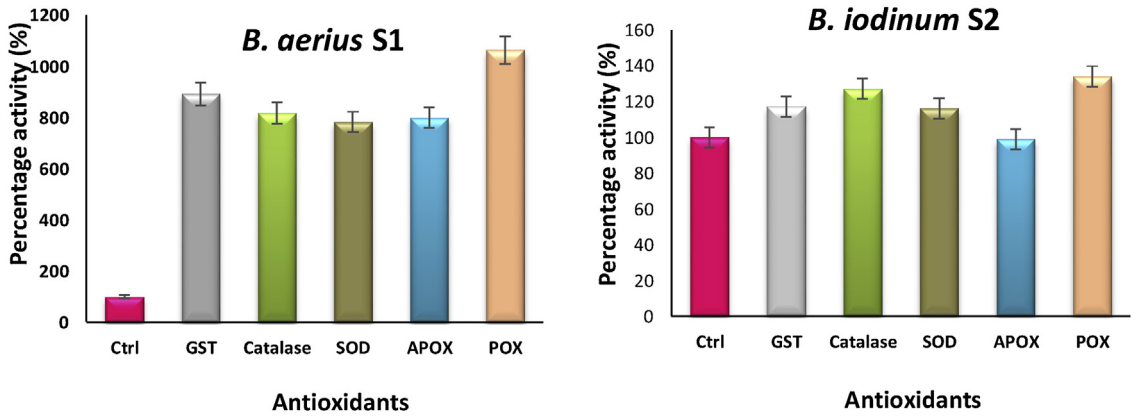

Fig. 2. Changes in Antioxidant enzymes activity profile, exhibited by *B. aerius* S1 and *B. iodinum* S2 upon exposure of 2 mM Cr $^{6+}$.

Table 1
Levels of reduced (GSH) and oxidized glutathione (GSSG), total glutathione, reduced and oxidized glutathione ratio, and nonprotein thiols in *B. aerius* S1 and *B. iodinum* S2 exposed to Cr^{6+} at 2 mM.

Bacterial strains	Cr Conc. (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
B. aerius S1	0	15.461	3.643	19.104	4.245	364.256	13.758	275.157
	2	19.104	8.412	27.516	2.271		16.509	
B. iodinum S2	0	15.934	7.082	23.016	2.250	663.913	17.704	177.043
	2	22.573	3.983	26.557	5.667		19.475	

determined in 10 l tannery effluent and change in Cr^{6+} was determined by Diphenylcarbazide method. It was clear that *B. aerius* S1 and *B. iodinum* S2 were capable of removing upto 99% Cr^{6+} from tannery effluent after 6 days of incubation when Cr^{6+} concentration was maintained at 2 mM (Table 2; Fig. 4).

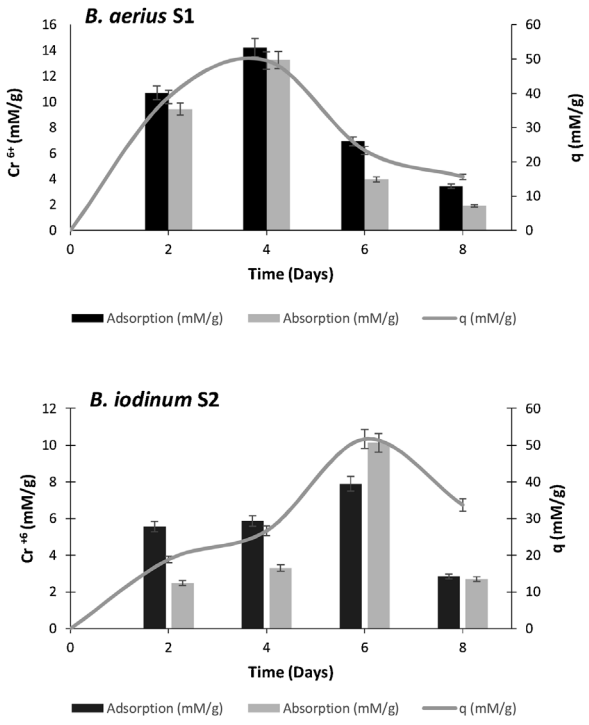

Fig. 3. Biosorption of Cr $^{6+}$ by *B. aerius* S1 and *B. iodinum* S2 at lab scale.

3.6. FTIR, SEM and EDX analysis

FTIR analysis of *B. aerius* S1 and *B. iodinum* S2 with and without chromium is shown in the Fig. 5. Infrared spectra of *B. aerius* S1 and *B. iodinum* S2, in the absence of any stress, exhibited characteristic absorption peaks of amino, hydroxyl, carboxyl, and sulphonate groups which confirmed the presence of corresponding groups on the cell surfaces. However, when the strains were subjected to heavy metal stress, changes in peaks and peak intensities were observed in the range of 3275 cm^{-1} and 1800–1000 cm^{-1} [24]. Under chromium stress, FTIR peaks of *B. aerius* S1 shifted from 1633 to 1628, 1535 to 1526, and 1052 to 1057. Also slight shift in hydroxyl group region was observed. Almost similar shift in the absorption peaks of similar regions was observed for the *B. iodinum* S2. Further confirmation of intracellular uptake of Cr^{6+} was done through SEM and EDX analysis which also confirmed the changes in cell state under heavy metal stress (Fig. 5).

4. Discussion

Biological procedures for the bioremediation of toxic heavy metals in the tannery effluents utilizing the potential

Table 2
Concentration of Cr^{6+} (μM) and the corresponding percentage reduction after regular intervals (2, 4, 6, 8 days) were determined in the original tannery effluent supplemented with 2 mM Cr^{6+} after treatment with *B. aerius* S1 and *B. iodinum* S2.

Bacterial strains	Days	2	4	6	8
S1 (Water)	Cr^{6+} (μM)	11.34	0.34	0.23	0.2
	% Reduction	**43**	**98**	**99**	**99**
S1 (Effluent)	Cr^{6+} (μM)	2.38	1.6	0.225	0.2
	% Reduction	**88**	**92**	**99**	**99**
S2 (Water)	Cr^{6+} (μM)	10.47	0.58	0.38	0.28
	% Reduction	**48**	**97**	**98**	**99**
S1 (Effluent)	Cr^{6+} (μM)	1.99	0.76	0.13	0.10
	% Reduction	**90**	**96**	**99**	**100**

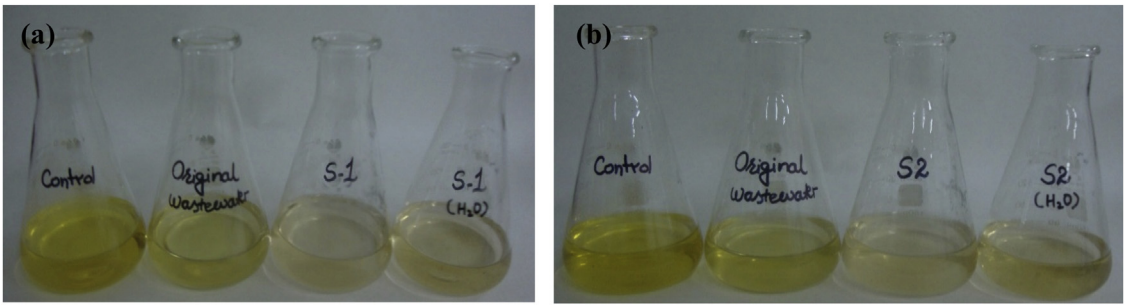

Fig. 4. Change in Cr^{6+} color with respect to control in flasks containing culture of bacterial isolates *B. aerius* S1 (a) and *B. iodinum* S2 (b) from the original tannery effluents.

microorganisms have been investigated by numerous researchers worldwide. Growing industrialization cause increased accumulation of Cr^{6+} to the alarming concentrations in the environment which pose serious environmental concern globally. The toxic nature of Cr^{6+} threatens all forms of life due to its teratogenic, mutagenic and carcinogenic properties. Therefore, there's an urgent need to reduce the concentration of Cr^{6+} in the biosphere to the permissible amount, determined by US-EPA *i.e.*, <0.05 mg L^{-1} [25].

A lot of work have been done on the utilization of Cr^{6+} resistant and reducing bacterial strains for the bioremediation purposes [26,27] under aerobic and anaerobic conditions [28,29]. Under chromium ion stress, Cr^{6+} resistant microbes utilize a number of strategies for their survival; consume a trace amount of metal ions for their metabolism; resist and/or detoxify excessive toxic amount of heavy metal present [30].

Intracellular Cr^{6+} reduction requires Cr^{6+} uptake. Once the chromate ions get entered into the cell, some of the ions get adsorbed on the outer surface (adsorption) before making its way into being accumulated inside the cell (absorption). Once accumulated, Cr^{6+} reduction into Cr^{3+} was carried out by the intracellular enzymes i.e., chromate reductases, where Cr^{3+} act as the terminal electron acceptor [6,31,32]. Thus, there are two key methods by which Cr^{6+} could be reduced into Cr^{3+} 1) hexavalent chromium bioaccumulation within the microbial cell 2) Cr^{6+} bioadsorption on the cell wall.

Adsorption leading to absorption of Cr^{6+} which is a necessary part of uptake process in the bacterial cell which ultimately leads to its reduction into less toxic Cr^{3+}, thus the atomic absorption spectroscopy clearly revealed the amount of Cr^{6+} being absorbed and adsorbed in the bacterial cells. The biosorption efficiency (q) of *B. aerius* S1 was as 39, 50, 23 and 16 mM Cr^{6+}/g while the Cr-biosorption efficiency (q) of *B. iodinum* S2 was as 19, 27, 52 and 34 mM Cr^{6+}/g after 2, 4, 6 and 8 days of incubation. After a certain time (6 days in our case), when the amount of Cr^{6+} is still exceeding, all the receptors of the bacterial cells get saturated, activating the efflux system for Cr^{6+} without reducing them.

In this study, two gram positive strains *Bacillus aerius* S1and *Brevibacterium iodinum* S2 was isolated from the local tannery wastewater samples; and the behavior of both of the strains were observed and compared under hexavalent chromium stress. Extensive research has been done on the use of chromate resistant *Bacillus* sp. for the bioremediation of Cr contaminated sites such as *Bacillus megatarium* TKW3; *Bacillus subtilis*; *Bacillus circulans*; *Bacillus cereus* and *Bacillus methylotrophicus* [6,11,30]. In contrast only few researchers investigate the potential of *Brevibacterium* sp. for the Cr bioremediation [33–36].

Generally, a potential microbe removes the heavy metal ions from the aqueous medium through biosorption or bioaccumulation or a combination of both processes. The initial passive uptake of metal ions occurs through biosorption which is followed by chemical bonding of Cr^{6+} to those sites on the cell surface which exhibit affinity for it. This bondage then leads to the reduction of Cr^{6+} into Cr^{3+} [37]. It has been suggested that Cr^{6+} ions exploit sulphate channels get an entry inside the cell where its reduction takes place [30,38], and the reduction of Cr^{6+} under aerobic conditions generate short lived lethal intermediates Cr^{5+} and Cr^{4+} that stimulate reactive oxygen species (ROS) production and subsequently cause toxicity in the cytoplasm [39].

Metal associated ROS generation promote oxidizing environment in the cell's vicinity, however, the cell's defense system comprising antioxidant compounds (superoxide dismutase, glutathione transferase, and catalase) could convert these biologically toxic species in to innocuous compounds [40]. Comparison of antioxidant profiles of *Bacillus aerius* S1 and *Brevibacterium iodinum* S2 under 2 mM Cr^{6+} stress showed very interesting results. Although Cr^{6+} stress in both strains generally provokes higher levels of antioxidant compounds, however the increase in *Bacillus aerius* S1 was substantially higher as compared to *Brevibacterium iodinum* S2 under the same stress conditions. In both strains, the highest increase was observed in POX which is 963 and 34% in *Bacillus* and *Brevibacterium*, respectively. Different environmental stress situations stimulate increased produced of peroxidases such as drought-stress, water stress [41], heavy metals stress (Cd, Cu, Al, Zn) [42], and gamma-radiation stress [40]. Suthar et al. [43] also have observed increased production of all the antioxidants under Cr^{6+} stress. Our results are also in good agreement with Lee and Shin [44] who observed increase in catalase activity under Cd stress. Increase in glutathione reductase activity was also reported by Lenartova et al. [45] and Khan et al. [23] under mercury and Cd stress, respectively. SOD induction was also reported by Lenártová et al. [45] under metal stress.

Wastewater offers a highly inhospitable environment for the propagation of nonindigenous bacteria as it also lacks essential nutrients required for the bacterial life support as well as it is rich in harmful compounds. Although, a lot of work has been done on the bioreduction trials of Cr^{6+} in LB media, however, only few replicate the same Cr^{6+} bioremediation trials in the original wastewater. Zahoor and Rehman [26] investigated Cr^{6+} bioremediation trial directly in original industrial effluent and demonstrated that *Bacillus* sp. JDM-2-1 and *Staphylococcus capitis* are capable of reducing Cr^{6+} (100 mg/l) upto 86% and 89%, respectively, after 144 h of incubation.

Biosorption due to nonspecific binding of Cr^{6+} or other heavy metal ions on the cell surface of the bacterial strain is highly dependent on the presence of functional groups on the active site of cell wall as well as physiochemical conditions of the solution. When Cr^{6+} interacts with the functional groups, changes in adsorption peaks were observed through FTIR analysis (Fig. 5). FTIR spectroscopy analysis of the untreated cells of *B. aerius* S1 and *B. iodinum* S2 showed presence of the functional group moieties on the cell surface, and changes in absorption peak intensities were

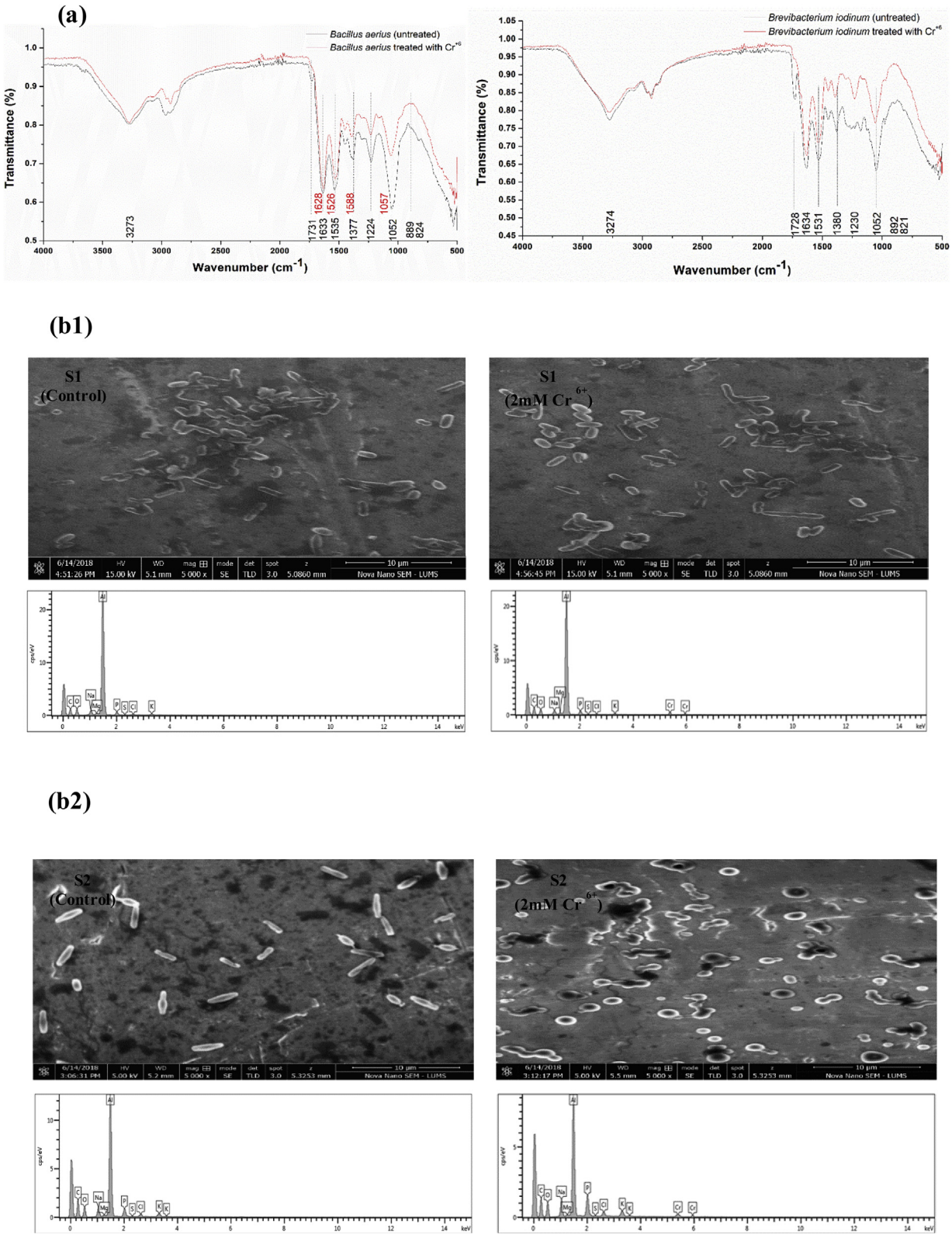

Fig. 5. (a) FTIR (b) Energy dispersive X-ray spectroscopy spectra analysis of *B. aerius* S1 (b1) and *B. iodinum* S2 (b2) in the presence and absence (control) of hexavalent chromium stress (2 mM Cr^{6+}).

observed after treatment with 2 mM Cr^{6+}. Our results are in good agreement with Lameiras et al. [46] and Pandi et al. [47].

Accumulation of Cr^{6+} within the cells of *B. aerius* S1 and *B. iodinum* S2 was determined through SEM/EDX analysis. Changes in cell morphology of both bacterial strains were observed after treatment with 2 mM Cr^{6+} (treated). SEM analysis of both *B. aerius* S1 and *B. iodinum* S2 showed changes in cell morphology after being challenged with 2 mM Cr^{6+} and EDX also confirmed Cr presence in the cell. Our results are in good agreement with Das et al. [48] and Khan et al. [23].

5. Conclusions

In the present study, two bacterial strains, *Bacillus aerius* S1 and *Brevibacterium iodinum* S2, showed maximum growth at 37 °C and pH 8. Both strains were able to resist Cr^{6+} upto 30 and 35 mM. Biosorption proficiency (q) of *B. aerius* S1 was 19, 27, 52 and 34 mM/g while for *B. iodinum* S2, it was 39, 50, 23 and 16 mM/g mM/g after 2, 4, 6 and 8 days of incubation. Cr^{6+} stress provoked significantly higher production of antioxidant enzymes (APOX, SOD, POX, GST, and CAT) in *B. aerius* as compared to the *B. iodinum*. Moreover, a significant GSH increase was determined in *B. aerius* S1 (364%) and *B. iodinum* S2 (663%) cultures under 2 mM Cr^{6+} stress as compared to the non-stressed cultures. Similarly, a rise in non-protein thiols was determined in *B. aerius* S1 (275%) and *B. iodinum* S2 (177%) under 2 mM Cr^{6+} stress. Pilot scale study demonstrated that both strains could reduce Cr^{6+} into Cr^{3+} within 6 days from the original tannery effluent with efficiency of 99%. Thus, both strains could be utilized to reclaim the chromium contaminated sites.

Conflict of interest

The authors have declared that no competing interests exist.

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