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Isolation, characterization, and multiple heavy metal-resistant and hexavalent chromium-reducing *Microbacterium testaceum* B-HS2 from tannery effluent

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ABSTRACT

A Cr^{6+} resistant *Microbacterium testaceum* B-HS2, isolated indigenously from tannery wastewater, showed optimum growth at 37 °C and pH 7. *M. testaceum* B-HS2 could resist to Cr^{6+} (48 mM) and heavy metals upto 2 mM (As^{2+}, Zn^{2+}, Cu^{2+}), 7 mM (Pb^{2+}) and 1 mM (Cd^{2+}, Ni^{2+}). Maximum activity of chromate reductase was achieved at 40 °C at pH of 7 and was inhibited in presence of all the heavy metals tested. *M. testaceum* B-HS2 biosorption efficiency (q) for Cr^{6+} was 31, 38, 66 and 47 mM/g after 2, 4, 6 and 8 days, respectively. Electron micrographs confirmed further the adsorption of metal leading to intracellular accumulation of Cr^{6+}. Functional groups such as amide and carbonyl moieties which actively participated in Cr^{6+} adsorption were determined through FTIR spectroscopy, and intracellular accumulation was also confirmed by energy dispersive X-ray (EDX) and scanning electron microscopy (SEM) analysis. Cr^{6+} presence triggers significant production of antioxidant enzymes [(Ascorbate peroxidase (APOX), Superoxide dismutase (SOD), Peroxidase (POX), Glutathione S-transferase (GST), and Catalase (CAT)]. Moreover, rise in glutathione and other non-protein thiol levels were determined which substantially neutralize Cr^{6+} generated oxidative stress. Pilot scale study revealed that *M. testaceum* B-HS2 was helpful in removing up to 96% Cr^{6+} from tannery effluent within 6 days and this microbial purified water is safe for the plant growth. Multiple heavy metal tolerance and high Cr^{6+} reduction potential make *M. testaceum* B-HS2 a candidate of choice to reclaim Cr^{6+} contaminated environment.
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1. Introduction

Rapid technological advancements and industrialization result in generation of huge amounts of hazardous waste material, having toxic heavy metals, when discharged indiscriminately into the environment causes serious health concerns (Hookoom and Puchooa, 2013). Chromium (Cr) has been extensively used in mining, metal plating, alloys manufacturing, wood preservation, tanning of hides and skins, pigments, glass and ceramics manufactures industries (Cheunga and Gu, 2007). Therefore, the build-up of anthropogenic inputs of Cr-containing wastes has reached at an alarming concentration during the past few decades that substantially pollute the arable soils and fresh water bodies. Cr toxic effects on the living species have been aggravated due to its mutagenic, carcinogenic, and teratogenic nature (Dhala et al., 2013).

Cr exists in several oxidation states, where Cr^{6+} is considered to be the most oxidized, toxic and reactive form, present predominantly in the natural aquifers (Dhala et al., 2013) because of its high water solubility. While Cr^{3+} being the less toxic, insoluble and environmental friendly, is primarily present in the municipal wastewater and has a strong affinity for the organic contaminants to form amorphous hydroxide complexes that easily get precipitated (Sawyer et al., 1994). Persistent nature of Cr^{6+} makes its anthropogenic release and subsequent accumulation a matter of serious environmental concern (Cheunga and Gu, 2007).

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Unlike organic contaminants, heavy metals are unable to be degraded through chemical or biological processes. Non-degradability and bioaccumulation character of the heavy metals pose a serious environmental concern, thus removing them through chemical or physical techniques are not only uneconomical, impractical and ineffective approach, but also generate huge amounts of toxic sludge. Thus, valence transformation is the only way of detoxification for the heavy metals, primarily valid for those metals that vary in toxicity when present in different valence states (Wu et al., 2010; Ayangbenro and Babalola, 2017). Utilization of microbes to bioremediate metal contaminated environment is an economical and environmental friendly approach. Thus, chromate reducing microbes, having the capability of chromium detoxification through valence transformation of Cr^{6+} to Cr^{3+} is considered to be suitable for on-site and *in situ* bioremediation (Mangaiyarkarasi and Geetharamani, 2014).

In the present study, Cr^{6+} bioremediation potential of *M. testaceum* B-SH2 from real tannery wastewater was ascertained. Antioxidant enzymes activities were evaluated by the bacterium to combat ROS produced under metal stress. The interaction of metal on the surface of bacterial cell and its subsequent intracellular accumulation was confirmed through FTIR, SEM and EDX analysis. Impact of Cr^{6+} on the growth pattern of bacterial strain, and any changes in proteomics and cell physiology were also determined.

2. Material and methods

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

Wastewater samples from tannery industries were collected aseptically from the various locations in Sheikhupura, and Qasoor, Lahore, Pakistan. Effluent samples were plated onto the modified Luria-Bertani (LB) agar amended with 1 mM Cr^{6+} 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 present in the medium (Fig. S1).

2.2. Bacterial identification

The isolate B-HS2 was characterized biochemically. For molecular characterization, genomic DNA of the selected strain was isolated according to Masneuf-Pomarède et al. (2007) and 16S rRNA gene fragment analysis was carried out by PCR amplification using universal bacterial primers RS1 (5-AAACTCAAATGAATTGACGG-3) and RS3 (5-ACGGGCGGTGTGTA-3) (Rehman et al., 2007). Fermentas purification kit (#K0513) was used to clean PCR product, and sequenced with Genetic analysis system model CEQ-800 (Beckman) Coulter Inc. Fullerton, CA, USA. The data obtained after sequencing was subjected to BLAST analysis.

2.3. Growth conditions of the bacterial isolate

The optimum growth conditions (temperature and pH) were determined according to Elahi and Rehman (2019). Effect of presence of Cr^{6+} on growth patterns of B-HS2 was scrutinized by growing bacterial strain with and without Cr^{6+} stress. Bacterial isolate B-HS2 was cultivated in LB broth, in mineral salt medium (MSM) broth [g/L: $FeSO_4{\cdot}7H_2O$ 0.015 g, KH_2PO_4 4.7 g, $MgSO_4{\cdot}7H_2O$ 1 g, $CaCl_2{\cdot}2H_2O$ 0.01 g, Na_2HPO_4 0.12 g, NH_4NO_3 4 g, $MnSO_4{\cdot}4H_2O$ 0.01 g, glucose 10 g and yeast extract 5 g (pH 7–7.2)] without Cr and MSM broth supplemented with 2 mM $K_2Cr_2O_7$ (experimental). The cell mass was determined by taking O.D at 600 nm with the interval of 4 h until 24 h of growth period.

2.4. Cross metal resistance

Minimum inhibitory concentration (MIC) value is regarded as the minimum concentration of metal able to inhibit bacterial growth. To determine MIC of metal ions, various concentrations of Cr^{6+} ($K_2Cr_2O_7$) and other metal heavy metal ion salts such as $CuSO_4{\cdot}5H_2O$, $CdCl_2$, $NiCl_2{\cdot}6H_2O$, $PbNO_3$, $NaAsO_2$ and $ZnSO_4{\cdot}7H_2O$ were separately added to 100 ml of MSM broth and were supplemented with 24 h old culture. These flasks were incubated under shaking conditions (150 rpm) at 37 °C in rotary shaker for 7 days. Growth was determined by taking absorbance at 600 nm.

2.5. Chromate reductase assay

2.5.1. Extra and intra-cellular enzyme assay

Crude enzyme was prepared by cultivating bacterial strain in 100 ml MSM medium supplemented with 2 mM $K_2Cr_2O_7$ and incubated under shaking conditions at 37 °C for 5 days. The cells were harvested at 6000 rpm for 10 min and solid ammonium sulphate was added to the supernatant until it is saturated up to 60%. The mixture was kept at −20 °C overnight for the precipitation. Extracellular proteins were centrifuged at 6000 rpm for 10 min and supernatant (upper 10 ml) was discarded while pellet with 3 ml of supernatant was used for extracellular enzyme estimation. Culture pellet was washed with phosphate buffer three times before sonication (15 pulses with a pause of 60 sec, three rounds). Sonicated pellet was subjected to centrifugation and the supernatant was employed for measuring the intracellular enzyme activity. Enzyme activities of extra and intra -cellular chromate reductase were evaluated according to Sarangi and Krishnan (2008).

2.6. Effect of pH, temperature, and metals on enzyme activity

Optimal pH for the activity of chromate reductase enzyme was assayed in buffers of different pH values (4.0–9.0). Each reaction contained 50 mM particular pH buffer with NADH (0.1 mM), Cr^{6+} (20 μM), and crude enzyme (100 μl), and incubated for 30 min. Chromate reductase activity was checked at different temperatures (30 °C–90 °C). Metals influence on the enzyme activity was determined by supplementing the reaction mixture with 0.1 mM concentration of metal ions (Na^+, Cu^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Hg^{2+}, and Zn^{2+}), in the reaction system. Enzyme activities were assayed according to Sarangi and Krishnan (2008).

2.7. Measurement of antioxidatants and glutathione contents

Antioxidants profiling of B-HS2 under Cr^{6+} stress was evaluated by growing strain in 100 ml MSM medium and incubated at 37 °C. Then broth was amended with Cr^{6+} stress (2 mM) after 24 h, and incubated again for a day. Bacterial cells were harvested by centrifugation at 14,000 rpm for 10 min, and weight of pellet was noted, and re-suspended in PBS before subjected to sonication. The sonicated pellet was centrifuged and the supernatant was used as antioxidant enzymes. Glutathione transferase (GST) was evaluated as mentioned by Habig et al. (1974). Peroxidase (POX) was assayed according to Reuveni et al. (1992) with minor modifications. Beers and Sizer (1952) method was used to determine catalase activity. Ascorbate peroxidase (APOX) activity was evaluated according to Israr et al. (2006) and Nakano and Asada (1987). The superoxide dismutase (SOD) activity was measured according to Ewing and Janero (1995).

Under chromium stress, the alteration in the production of glutathione (GSH) and non-protein thiols (NPSHs) was estimated according to Khan et al. (2015). Briefly, three flasks of culture were prepared and 2 mM $K_2Cr_2O_7$ stress was given in two flasks after 24 h, and third flask acted as control because no metal was added

in this flask. All the flasks were again incubated for another 48 h. Culture was harvested, washed thrice with 1 mM PBS, weighed and re-suspended in 5% sulfosalicyclic acid (1 ml). Pellet was broken down by sonication, followed by centrifugation at 14,000 rpm for 10 min at 4 °C and aliquot was divided into two equal parts. One part was used to assess GSH level and other part was used to estimate NPSHs level. The amount of GSH, oxidized glutathione (GSSG) and NPSHs was quantified by Khan et al. (2015).

2.8. Metal processing ability of the bacterial isolate

The ability of metal processing of the bacterium was evaluated by measuring changes in the quantity of Cr^{6+} in the culture medium by atomic absorption spectrophotometer (Zahoor and Rehman, 2009; Elahi and Rehman, 2019).

2.9. Chromate reduction in tannery effluent

The Cr^{6+} reduction potential of B-HS2 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 1.5 L 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 intervals (2, 4, 6, and 8 days of incubation). Cells were harvested at 4000 rpm for 10 min and supernatants were used for the estimation of Cr^{6+} by Diphenylcarbazide method. The calibration curve, established under the same experimental conditions using standards of Cr^{6+} solution, was used to calculate any change in the Cr amount before and after bacterial treatment.

2.10. Effect of microbial treated wastewater on Vigna radiata

Effectiveness of microbially treated tannery effluent was ascertained for the plant cultivation. In this, autoclaved soil was used to prepare small pots and *Vigna radiata* (mung beans) seeds were sown into each pot. The experimental pot was watered with the treated effluent while watering of control plants were done with either tap water or untreated (original) wastewater. Seeds were allowed to grow under 1:1 light and dark period for 10 days. To determine the effects of toxic Cr^{6+} metal on plant growth, changes in plants growth with bacterially purified wastewater and untreated wastewater, was observed.

2.11. Bacterial protein profiling though SDS PAGE

Bacterial isolate was cultures in MSM liquid medium in the presence and absence of 2 mM Cr^{6+}. The samples were prepared and SDS-PAGE was done according to Laemmli (1970).

2.12. FTIR, SEM and EDX analysis

FTIR spectroscopy was employed to obtain Infrared spectra for B-HS2 under Cr^{6+} stress. Specimens were treated as mentioned by Deokar et al. (2013). In order to investigate the mechanism of metal-microbe interaction, it is vital to confirm the presence of chromium species (Cr^{+3}, Cr^{6+}) within the microbial cell and for this purpose EDX analysis was done.

For scanning electron microscopy, bacterial samples were treated as mentioned by Khan et al. (2016). 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. (2016). 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.

2.13. Statistical analysis

Each experiment 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. Physiochemical characteristics

Temperature of the effluent samples ranged from 22 to 34 °C, and pH range was 6.0–10.0. The colonies appeared on the LB agar plates containing 2 mM Cr^{6+} were further streaked on higher concentrations of Cr^{6+}. The isolate that resisted highest Cr^{6+} concentration (48 mM) was selected and named as B-HS2.

3.2. Biochemical and molecular characterization of bacterial isolate

B-HS2 is a gram positive strain and its biochemical characteristics are mentioned in Table S1. Molecular identification through 16S ribotyping showed that it shares 91% homology with *Microbacterium testaceum* (Accession number JQ916902). The phylogenetic tree of *M. testaceum* was constructed to determine its similarity with other *Microbacterium* strains using MEGA7 program taking bootstrap value 500 (Fig. S2).

3.3. Optimization of growth conditions

M. testaceum B-HS2 optimum growth was determined as 37 °C and pH 7. *M. testaceum* B-HS2 growth was substantially declined in presence of Cr^{6+} as compared to the culture containing no Cr^{6+} (Fig. 1).

3.4. Cross metal resistance

M. testaceum B-HS2 could resist Cr^{6+} upto 48 mM and also capable of tolerating other heavy metals, viz., 7 mM (Pb^{2+}), 2 mM (Zn^{2+}), 2 mM (Cu^{2+}), 2 mM (As^{3+}), 1 mM (Cd^{2+}), and 1 mM (Ni^{2+}). Resistance order according to metal ions concentration was $Cr^{6+} > Pb^{2+} > As^{3+}/Zn^{2+}/Cu^{2+} > Cd^{2+}/Ni^{2+}$.

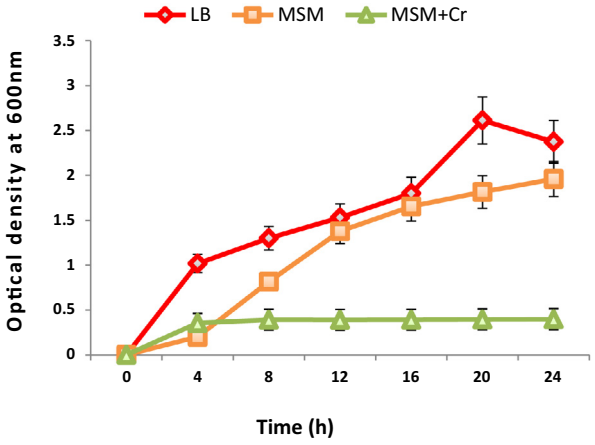

Fig. 1. Growth curves of *M. testaceum* B-HS2 in LB medium (control), mineral salt medium (MSM), and MSM supplemented with 2 mM $K_2Cr_2O_7$ (treated) incubated at 37 °C. Optical density was taken at 600 nm after regular time interval.

3.5. Characteristics of chromate reductase

Chromate reductase of *M. testaceum* B-HS2 showed optimum activity at pH 7.0 (Fig. 2a) and at 40 °C (Fig. 2b). All the heavy metals used inhibit the enzyme activity (Fig. 2c).

3.6. Quantification of antioxidants enzymes, glutathione and Non-protein thiols

Cr^{6+} presence stimulates activities of all the antioxidative enzymes. Significant increase in POX (86%) and CAT (42%) activities was estimated, however, a relatively less increase in activities of APOX (19%), SOD (28%), and GST (14%) was also determined (Fig. 2d). Cr^{6+} stress also stimulates GSH and NPSHs levels in *M. testaceum* B-HS2 (Table S2). In the presence of 2 mM Cr^{6+}, 326% increase in GSH was determined as compared to the control. An increase in non-protein thiols was also determined (122.25%) in metal stressed culture when compared with the non-metal stressed culture.

3.7. Cr^{6+}bioprocessing potential of B-HS2

3.7.1. Biosorption of Cr^{6+}

Biosorption potential of *M. testaceum* B-HS2 was assessed by cultivating it in LB broth supplemented with 2 mM Cr^{6+} (Fig. 3a). Biosorption proficiency (q) of *M. testaceum* B-HS2 was determined after 2, 4, 6 and 8 days which was 31, 38, 66, and 47 mM/g, respectively. Cr^{6+} was also found within bacterial cells i.e., 13, 17, 27 and 12 mM/g, after 2, 4, 6, and 8 days respectively. While 9, 14, 29 and 26 mM/g of Cr^{6+} got adsorbed on the cell surface after 2, 4, 6, and 8 days, respectively.

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

M. testaceum B-HS2 bioremediation potential was tested at pilot scale, when the efficiency of the isolate was determined in 10 L tannery effluent supplemented with a specific concentration of Cr^{6+} and then reduction in Cr^{6+} was confirmed by Diphenylcarbazide method. It was clear that bacterium was capable of removing upto 88 and 96% Cr^{6+} after 3 and 6 days of incubation from the real tannery effluent where 2 mM Cr^{6+} concentration was maintained (Fig. S3). The change in color, with respect to control (no bacterium), in flask containing tannery effluent and culture of *M. testaceum* B-HS2 is shown in Fig. S4.

3.8. Phytotoxicity study

The phytotoxicity experiment was conducted in order to determine any change in toxicity of the original wastewater post bacterial treatment. The setup consist of control 1 (water containing 2 mM Cr^{6+}), control 2 (wastewater containing 2 mM Cr^{6+}), and

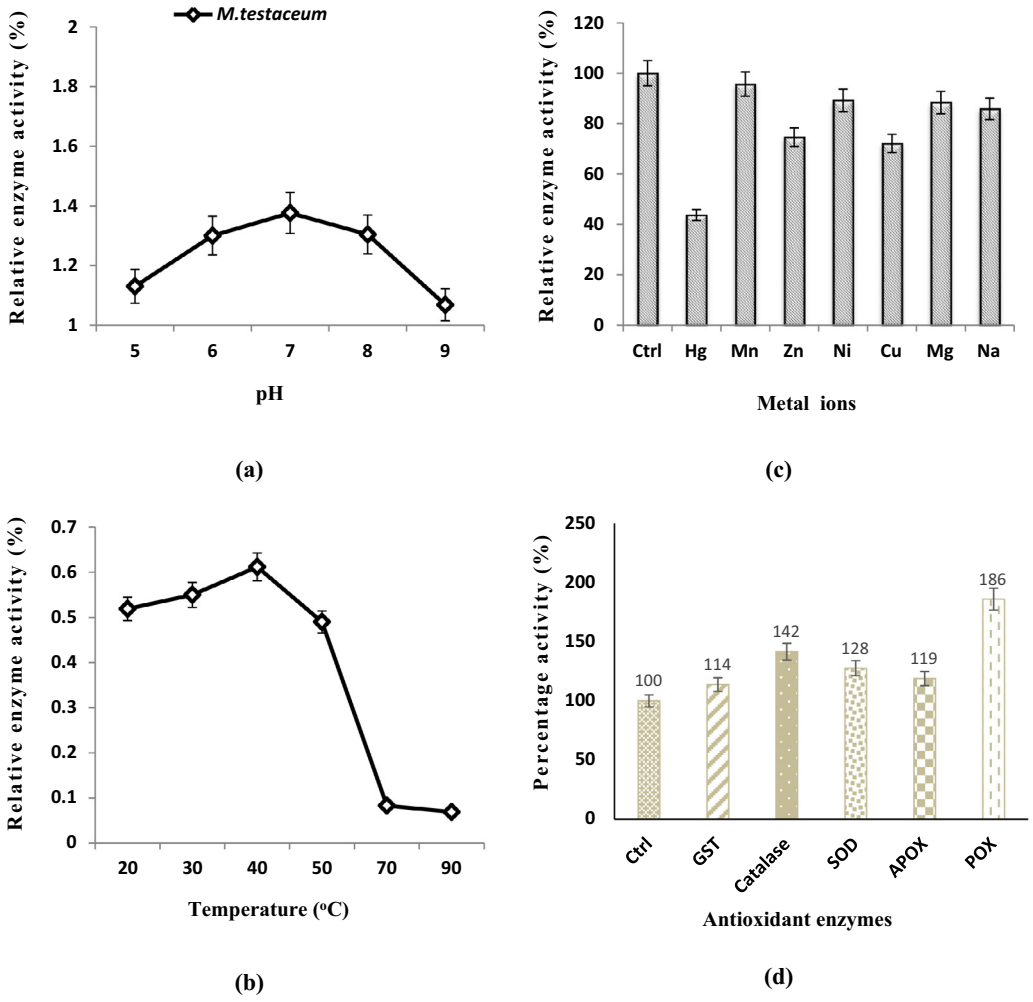

Fig. 2. Effect of pH (a), temperature (b), metal ions (c) on the chromate reductase activity and changes in antioxidant enzymes activity profile (d), exhibited by *M. testaceum* B-HS2 upon exposure of 2 mM Cr^{6+}.

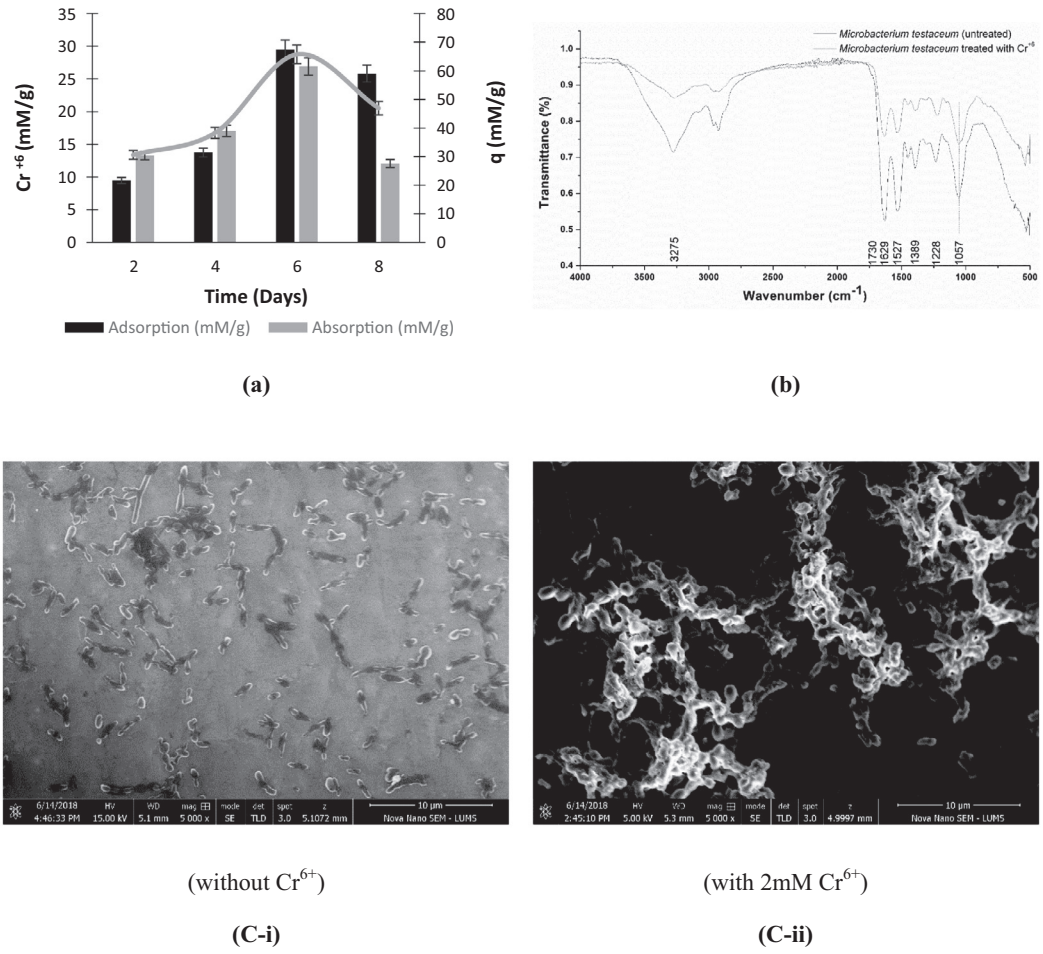

Fig. 3. (a) Biosorption of Cr^{6+} by *M. testaceum* B-HS2 at lab scale incubated for 8 days. (b) FTIR spectra of *M. testaceum* B-HS2 in the presence and absence (control) of hexavalent chromium stress (2 mM). (c) Images of scanning electron microscopy of *M. testaceum* B-HS2 in the absence (C-i) and presence (C-ii) of Cr^{6+} (2 mM).

treated wastewater (wastewater containing 2 mM Cr^{6+} also supplemented with the bacterial culture *M. testaceum*-B-HS2), and all the three containers were incubated for 6 days at room temperature. The results clearly showed that the Cr^{6+} concentration remained unchanged in control 1 and 2, while in treated container, *M. testaceum*- B-HS2 has potentially removed 99% of Cr^{6+}, clearly demonstrated by change in color and also confirmed by estimation through diphenylcarbazide method. The treated and control samples were then utilized for the cultivation of seeds of *Vigna radiata*. Thus phytotoxicity study results revealed that the treated tannery effluent can be safely used for the growth of plants as demonstrated by the decent germination of *Vigna radiata* when watered with the bacterially processed tannery wastewater as compared to the control (original wastewater). The toxicity of original wastewater get highly reduced after bacterial treatment accompanied by the reduction of noxious toxic Cr^{6+} (Fig. S5).

3.9. Protein analysis

Presence of Cr^{6+} in the medium imposes changes in cell physiology and proteomics. In this regard protein profiling of *M. testaceum* B-HS2 was ascertained in absence (control) and presence of 2 mM Cr^{6+} (treated) by SDS-PAGE. Cr^{6+} presence trigger over expression of some of the proteins and some proteins get diminished. For instance an approximately 65 kDa protein is present in control culture sample while it disappeared under stress (2 mM Cr^{6+}). Low molecular weight protein bands (20 and 16 kDa) indicate the induction of metallothioneins under Cr^{6+} stress (Fig. S6).

3.10. FTIR, SEM and EDX analysis

FTIR analysis of *M. testaceum* B-HS2 with and without chromium is shown in Fig. 3b. Infrared spectra of *M. testaceum* B-HS2, when grown normally, showed characteristic absorption peaks of amino, hydroxyl, carboxyl, and sulphonate groups ascertained their existence on cell surface. However, when bacterium was challenged with 2 mM Cr^{6+}, alteration in peaks were detected in the region of 3275 cm^{-1} and 1800–1000 cm^{-1} (Bennett et al., 2010). FTIR results revealed that when chromium first interacts with the cell surface, it gets sequestered on the surface via adsorption. This could be a result of surface complexation of chromium with the functional groups present on the cell wall. As a result, changes in the absorption peaks of the spectrum were witnessed. SEM (Fig. 3ci–ii) and EDX analysis (Fig. S7) also support the evidence of intracellular accumulation of Cr^{6+}.

4. Discussion

Persistence and non-degradability of heavy metals make bioremediation popular among the researchers globally. Chromium toxic nature causes many environmental and global health issues when discharged directly into the biosphere. Upon interaction with

Cr, microbes could only consume its trace amount for their metabolic functioning and remain resistant to the huge levels of this heavy metal. Numerous studies have shown the isolation of hexavalent chromium resistant and reducing bacteria from Cr-polluted and non-polluted sites (Zhang et al., 2013; Fan et al., 2018) of different environmental conditions i.e. aerobic as well as anaerobic condition (Pattanapipitpaisal et al., 2001; Wani et al., 2007).

This study reports the isolation of *M. testaceum* B-HS2 from tannery effluent sample that is capable of resisting hexavalent chromium upto 48 mM. Hexavalent chromium resistant *Microbacterium* species have already been reported (Table S3) by many

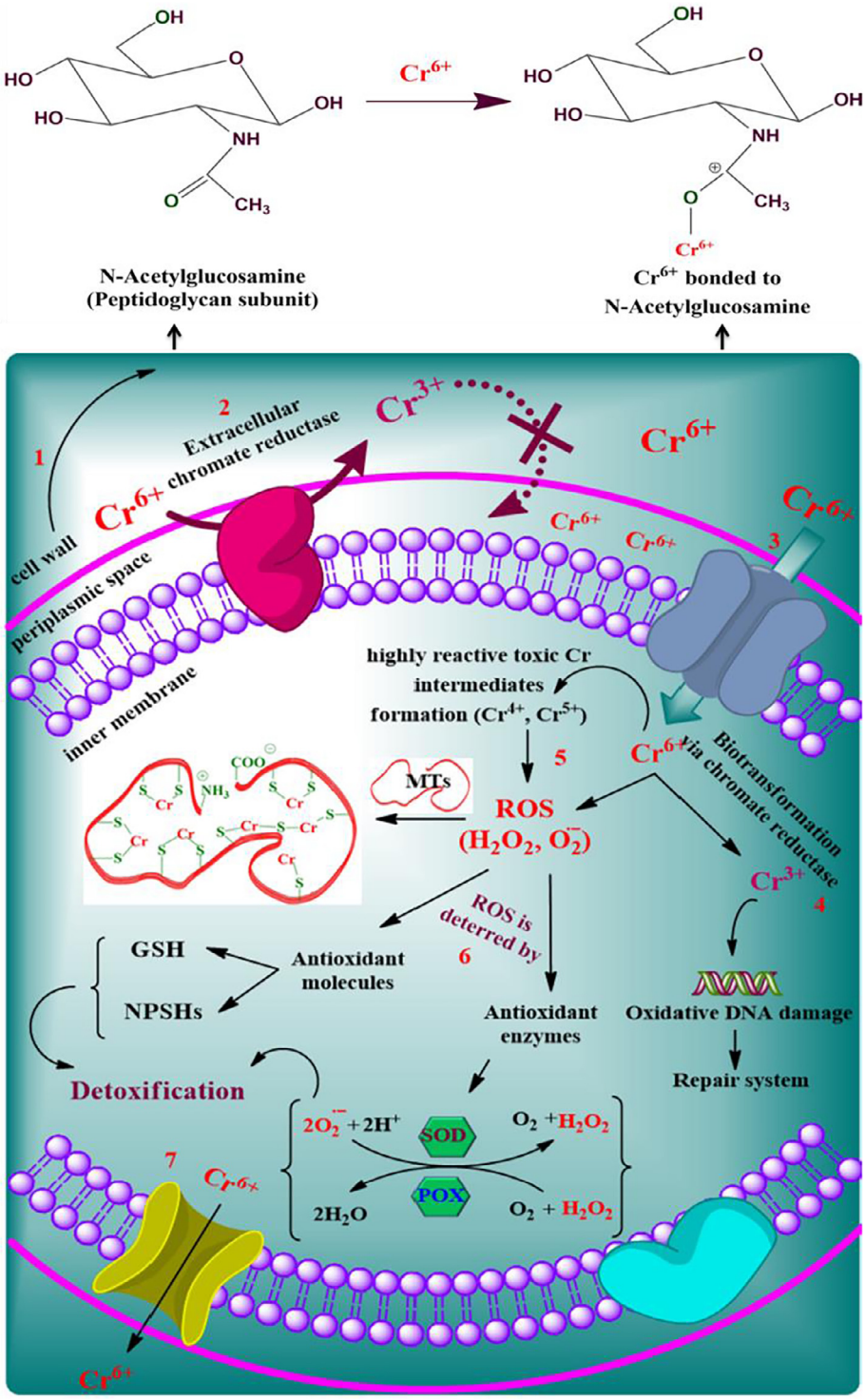

Fig. 4. Proposed Cr^{6+} resistance and reduction mechanism in gram positive bacterium, *M. testaceum* B-HS2. (1) Chitin is the primary site on bacterial surface that adsorbs Cr^{6+} in monolayer. (2) Cr^{6+} reduces into Cr^{3+} extracellularly by chromate reductase. (3) Cr^{6+} enters into cell cytoplasm and changes into Cr^{3+} by intracellular chromate reductase. (4) Cr^{3+} is involved in DNA damage. (5) Some portion of Cr^{6+} changes into its intermediate species i.e. Cr^{5+} and Cr^{4+} which are involved in the generation of ROS. (6) The oxidative stress resulted by the formation of ROS is combated by two ways; antioxidant enzymes and antioxidant molecules. (7) After certain level of accumulation Cr^{6+} expel out through efflux system.

researchers (Cheunga and Gu, 2007; Liu et al., 2012; Focardi et al., 2013; Henson et al., 2015).

Bioremediation generally comprises two phases through which a potential microbe removes metal from aqueous solution; initial rapid passive metal uptake through adsorption, ion-exchange, chelation (Bharagava and Mishra, 2017) leading to slow chemical bonding of Cr^{6+} to high affinity sites. This is followed by the reduction of Cr^{6+} into Cr^{3+} when Cr^{6+} acts as terminal electron acceptor before it makes complex with the cell wall (Cheunga and Gu, 2007).

Chromate oxyanions actively transverse biological membranes through sulphate channels, taking advantage of the chemical analogy they share with the sulphate ions (Cervantes and Campos-Garcia, 2007). As soon as Cr^{6+} enters the cell, under aerobic conditions it rapidly reduces itself into short lived noxious intermediates Cr^{5+} and Cr^{4+} toxicate the cytoplasm (Cheunga and Gu, 2007; Bharagava and Mishra, 2017). However, enzymatic and non-enzymatic mechanisms are responsible for the conversion of Cr^{6+} into Cr^{3+} aerobically or anaerobically. Though, it is not confirmed that these conversions (Cr^{5+} to Cr^{4+}/Cr^{4+} to Cr^{3+}) are either spontaneous or enzyme mediated, however, electron from the endogenous reserve and from NADPH, NADH are involved in Cr^{6+} reduction (Bharagava and Mishra, 2017).

AAS analysis revealed that the maximum metal processing potential of *M. testaceum* B-HS2 was attained after 6 days where it removes 66% of Cr^{6+} from the medium. Furthermore, pilot scale experiment also replicates the outcomes as the maximum Cr^{6+} removal (96%) from the real tannery effluent was obtained after 6 days of incubation. This microbially treated tannery effluent was further used for watering purpose in seed germination and the seeds watered with the microbially purified water exhibited a significant rise in plant height in comparison with the seeds treated with the original tanner effluent. The plants growth was delayed and hindered in untreated wastewater, thus the microbially purified wastewater can safely be used for crops irrigation purposes in real time setup (Fig. S6).

Heavy metal ions have been reported to promote increased generation of reactive oxygen species (ROS) (Kappus, 1987) imbalancing the cell's reduced environment and in this case, one electron shuffling between Cr^{5+} and Cr^{6+} generates ROS buildup, which complexes with cellular materials like DNA, lipids and proteins, results in malfunctioning (Cervantes et al., 2001; Cheunga and Gu, 2007). Ackerley et al. (2004), and Hussein and Joo (2013) reported that ROS is mainly combated by the antioxidant compounds (superoxide dismutase, glutathione transferase, and catalase), which impede the biologically toxic species by converting them to innocuous compounds (Fig. 4).

Our results showed that Cr^{6+} generally induces elevated production of all the antioxidants; however, substantial increase in CAT (42%) and POX (86%) activities was noticed as compared to the GST (14%), APOX (19%), and SOD (28%). Higher production of peroxidase has been reported in different stress conditions i.e., drought-stress, water stress (Zhang and Kirkham, 1994; Chaoui et al., 1997), gamma-radiation (Hussein and Joo, 2013) and also under metals stress (Cd, Cu, Al, Zn) (Hussein and Joo, 2013). The outcomes of the current study are agreed with Chen et al. (2000) who reported higher peroxidase production under Cu stress. An increase in catalase activity was also documented in the presence of Cd (Lee and Shin, 2003). Our findings are also in good agreement with Khan et al. (2016) and Lenártová et al. (1998) who reported a higher induction of glutathione reductase under Cd and mercury stress, respectively.

Interaction of Cr^{6+} (adsorption and/or absorption) with the functional groups on the bacterial cell surface was confirmed through FTIR analysis. FTIR profile of control displayed various peaks indicating the complexity of bacterial cell surface. However, when the cells were treated with Cr^{6+}, changes in band intensities were observed. Hydroxyl and amide groups stretching were observed at 3275 cm^{-1} and 16290 cm^{-1}, respectively. Shift in peaks and change in peak intensities revealed the participation of carbonyl, carboxyl, amino and hydroxyl groups in metal binding. Lameiras et al. (2008) and Pandi et al. (2009) also reported the metal interaction with functional groups by FTIR analysis. To confirm the presence of Cr^{6+} within *M. testaceum* B-HS2, surface characterization was done by SEM/EDX analysis. SEM analysis revealed that control cells showed a smooth and regular surface and EDX also confirmed Cr absence in the cell. The outcomes of the present work are in good agreement with Khan et al. (2016).

5. Conclusions

A Cr^{6+} resistant (48 mM) *M. testaceum* B-HS2, grew well at 37 °C and pH 7, could resist other heavy metals upto 2 mM (As^{2+}, Zn^{2+}, Cu^{2+}), 7 mM (Pb^{2+}) and 1 mM (Cd^{2+}, Ni^{2+}). The optimum enzyme activity was measured at 40 °C and pH of 7. Cr^{6+} treated *M. testaceum* B-HS2 showed significant increase in the production of antioxidant enzymes. Moreover, elevated levels of GSH and NPSHs substantially neutralize Cr^{6+} generated oxidative stress. Pilot study revealed that *M. testaceum* B-HS2 was helpful in removing Cr^{6+} upto 96% from the real tannery effluents within 6 days of incubation and this microbial purified wastewater can at least be used for crops irrigation purposes. Thus, it could be a promising candidate for the amelioration of industrial effluents contaminated with toxic heavy metal ions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2019.02.007.

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