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Evaluation of performance of *Planococcus* sp. TRC1 an indigenous bacterial isolate monoculture as bioremediator for tannery effluent

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ABSTRACT

An indigenous bacterial isolate *Planococcus* sp. TRC1 was found to tolerate Cr(VI) solution up to 500 mg/L concentration when grown in mineral salt media. Gene sequencing of the isolated strain using 16S rDNA technique and phylogenetic analysis confirmed that the species was 96% close to *Planococcus maritimus* (KP8). Studies on cell dynamics in batch bioreactor showed the maximum specific cell growth rate (μ_{max}) to be 0.276 h^{-1}. Removal of Cr(VI) was observed to be dependent on initial chromium concentration and the maximum removal of Cr(VI) (75 ± 3%) was obtained at 25 mg/L Cr(VI) solution for an incubation period of 72 h. Above this value, the removal of Cr(VI) was declined. Scanning electron microscope and Fourier transform infrared spectroscopic studies indicated that the Cr(VI) removal took place by the adsorption process on the cell outer membrane. It is observed that the adsorption process follows the Freundlich adsorption isotherm (R^2 = 0.987). *Planococcus* sp. TRC1 reduced 80 ± 5% chemical oxygen demand (COD) of tannery effluent (COD 7,270 ± 45 mg O_2/L) within 48 h of batch treatment. The elimination of cytotoxicity and genotoxicity imparted by the raw tannery effluent and Cr(VI) solution after bacterial treatment is an important observation which reflects the eco-friendly behavior of the *Planococcus* sp. TRC1 mediated tannery effluent and aqueous Cr(VI) solution treatment. The results revealed the applicability of *Planococcus* sp. TRC1 in both tannery waste and Cr(VI) reduction without affecting our environment.

Keywords: *Planococcus* sp. TRC1; COD reduction; Chromium(VI) reduction; Ecotoxicity

1. Introduction

Tannery effluent released by leather industries is of great concern to the environment because of its high chemical oxygen demand (COD) value even after treatment by conventional physicochemical methods. Although it is well known that Cr(VI) is a great threat to living bodies due to its carcinogenic, mutagenic, and teratogenic effects [1,2], interestingly, apparently

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environment-friendly Cr(III) coming out with the tannery effluent produces toxic effect on natural flora and fauna [3,4]. This ambiguous behavior of Cr(III) is due to its oxidation to Cr(VI) state absorbing UV-ray from sunlight. Chromium has now been identified as the second most common inorganic contaminant of ground water at hazardous waste sites and a known potential genotoxicant to plants and animals [5,6]. Intracellular-reduced Cr(VI) has the capability to produce genetic lesions by interacting with the DNA due to which DNA adduct, DNA strand break, and DNA protein crosslinks are generated [7]. The various conventional treatments which include precipitation, ion exchange, evaporation, reverse osmosis, and electrochemical treatment for Cr(VI) removal [8] possess certain specific drawbacks with respect to cost, sludge production, toxic intermediate, and high input of energy [9]. Moreover, none of these processes can meet the Cr(VI) discharge concentration set by different statutory bodies of government agencies looking after environmental protection. The search for a highly efficient treatment process is therefore in demand.

The biological treatment processes can be the useful choice, as they are economical with low energy input and eco-friendly. More importantly biological processes can reduce Cr(VI) concentration to the extent which is much below the discharge standard set by regulating authorities. It has been reported that a number of micro-organisms viz., *Thiobacillus ferrooxidans* [10], *Bacillus flexus* [4], halo, meso, thermophilic bacteria [4,11], and many others can efficiently reduce high COD levels of industrial wastewater. It is reported that a number of micro-organisms viz., *Pseudomonas* sp. [4], *Bacillus* sp. [12], *Bacillus cereus* [13], *Acinetobacter* [14] and *Ochrobacterium* sp. [15], and many others can reduce the toxic form of Cr(VI) into its less toxic Cr(III) form. These micro-organisms, however, are very specific in nature and are tailor made for a specific biodegradation process. The recent trend in research is to search for micro-organisms that can be utilized for multi-task operations. In the field of Cr(VI) biodegradation process, more emphasis is now paid to search for a specific micro-organism which cannot only degrade Cr(VI) to Cr(III) but should be able to degrade simultaneously other toxic materials viz., phenolic compounds present in the industrial wastewater.

In the present investigation, an attempt has been made to isolate, identify, and characterize a suitable micro-organism from tannery effluent which is capable to remove Cr(VI) and reduce COD from tannery waste effluent. In a separate experiment, suitability of the isolated cell for degrading phenolic compounds has also been tested. An attempt has also been made to study in detail about the Cr(VI) removal mechanism by the isolated cells. The eco-friendly nature of the treatment process has been tested using *Allium test*.

2. Materials and methods

2.1. Materials

Nutrient broth, nutrient agar (Hi Media Chemicals, India), potassium dichromate ($K_2Cr_2O_7$), 1,5-diphenylcarbazide, acetone, acetic acid, KH_2PO_4, K_2HPO_4, NH_4Cl, $MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}2H_2O$, $FeCl_2{\cdot}4H_2O$, $MnCl_2{\cdot}4H_2O$, $Na_2MoO_4{\cdot}2H_2O$, glucose, tris-buffer, acetocarmine, sodium hypochlorite, and analytical grade chemicals from Merck, India were used. Others such as Di-sodium salt of EDTA, Ethidium Bromide (EtBr), and ethyl methane sulfonate were purchased from Sigma Aldrich, USA. All other chemicals were of analytical grade.

2.2. Collection of tannery effluent and its characterization

Effluent samples were collected from tannery industrial area of Kolkata, West Bengal, India that use chromium for leather tanning. The samples were stored at 4˚C for various experimental works. Physico-chemical characterization using standard protocol was done [16]. For pH (Metler Toledo), color, turbidity, and salinity specific probes of Inolab analyzer were used. Standard DPC method and barium sulfate titration method for Cr(VI) and sulfate were used [16]. The COD was estimated following standard reflux method using COD analyzer digester (Lovibond, Germany).

2.3. Screening for Cr(VI) resistant bacteria and acclimatization

The culturable bacterial species present in tannery wastewater were isolated using standard serial dilution method. In nutrient agar, three bacterial colonies with specific color such as yellow, cream, and orangish red were obtained after 48 h of incubation at pH 7.0 and 30˚C. All the three bacterial colonies obtained were purified as single cell colony in nutrient agar plates and designated as TY (tannery yellow), TR (tannery orangish red), and TC (tannery cream). When screened for resistance to Cr(VI), only one bacterial isolate designated as TRC1 (tannery red color colony) showed well tolerance to 100 mg/L Cr(VI), in both minimal salt agar media and nutrient agar plate at pH 7.2 [17]. This specific Cr(VI)-tolerant bacteria (TRC1) was further acclimatized in increasing concentration of

Cr(VI) and in tannery wastewater to check its bioremediation potential.

2.4. Characterization and identification of bacterial isolate

The gram staining, colony characteristics, and biochemical tests (IMViC tests) for the specific selected bacterium TRC1 were carried out using established protocols [18,19]. Molecular identification using 16S rDNA analysis has been done from Chromas Biotech Pvt. Ltd, Kolkata, India. The sequence alignment was performed using blastn to identify the genus [20]. The Clustal W program was followed for similarity analysis with the best matched sequences retrieved from the database [http://ncbi.nlm.nih.gov].

2.5. Biodegradation potential of isolated bacterial strain TRC1 for tannery waste and Cr(VI)

The COD reduction from tannery effluent and Cr(VI) removal from synthetic aqueous media were examined using the Cr(VI) resistant isolate (TRC1) in two separate batch experiments. Separately to 100 ml of tannery effluent and 100 ml mineral salt media (MSM) containing various concentration of $K_2Cr_2O_7$ as Cr(VI) in 500 ml flask, added 10% inoculum volume of acclimatized strain of *Planococcus* sp. TRC1 and kept for various time periods at 30˚C, pH 7.0, and rpm 120 in BOD incubator shaker. At specified time, both bacterial-treated and untreated samples were centrifuged at 6,000× *g* for 10 min (Eppendorf centrifuge 5810R) and filtered using Wattman-42 to estimate the remaining COD and Cr(VI) in the respective supernatants by automatic COD analyzer digester (Lovibond, Germany) and UV/Vis spectrophotometeric method using 1,5-diphenylcarbazide [16] at 540 nm, respectively. The washed, PBS-resuspended pellet's absorption at 600 nm was checked for growth kinetics of this bacterial isolate. To estimate the total chromium, Cr^{3+}, the tannery industrial wastewater, was oxidized to Cr^{6+} using potassium permanganate [21]. The optimization of experimental process parameters were carried out at fixed Cr(VI) concentration with varying experimental condition such as pH, temperature, rpm, and % inoculum.

2.6. SEM analysis

For scanning electron microscope (SEM) the *Planococcus* sp. TRC1 grown in the presence of 25 mg/L Cr(VI) was collected by centrifugation. The cell pellet was suspended in sterile PBS buffer and a drop was placed on a cover slip, air dried, and immersed in glutaraldehyde (2.5% v/v) at room temperature and washed thoroughly with sterilized PBS. The cells were dehydrated by passing through the increasing concentration of ethanol, mounted on an electron microscope stub and coated with gold before they were examined by SEM (JEOL JSM-5800).

2.7. FTIR analysis

To investigate the bacterial cell surface functional groups involvement, the overnight grown bacterial culture in the presence and absence of Cr(VI) was harvested by centrifugation at 6,000× *g* at 4˚C for 20 min. Using the general protocol [14,22], washed and harvested cell pellet was dried at 50˚C, ground with potassium bromide (KBr) and analyzed using FTIR spectroscopy with Perkin Elmer (software-spectrum RX1) instrument.

2.8. Ecotoxicity test

Standard procedure of *Allium cepa* (*A. cepa*) root growth inhibition and chromosomal aberration test was followed with some modifications [23] to check the safety of the biotreatment of Cr(VI) solution (25 mg/L) and tannery waste effluent (100%) by the isolated strain *Planococcus* sp. TRC1 with respect to its untreated control. The onion bulbs after proper processing and growing in tap water for 24 h were exposed to bacterial treated and untreated tannery wastewater and aqueous Cr(VI) samples for 24 h in a plant growth chamber at 23–25˚C maintaining dark and light condition. The changes in onion root length were measured after 24 h exposure. The conventional Feulgen squash method was followed for the chromosome aberration study [24]. Chromosomal aberration and % mitotic index (MI) were scored, in 100 anaphase–telophase cells per onion, corresponding to 500 cells per experimental condition. These experiments were conducted in triplicates at each experimental point.

3. Results and discussion

3.1. Characterization of tannery industry wastewater

The physicochemical characteristics of the selected tannery effluent were analyzed and summarized in Table 1(a). The results obtained indicated that COD, TDS, sulfate, and total chromium concentration were much higher with respect to the permissible level set by all the regulatory authorities. Results evidently

Table 1a
Physicochemical characteristics of tannery industry wastewater

Parameters	Values
Odor	Obnoxious
Temperature (°C)	30 ± 02
pH	5.4 ± 01
COD (mg O_2/L)	7,270 ± 45
Conductivity (μS/cm)	4.4 ± 0.98
Total dissolved solid (mg/L)	5,710 ± 53
Salinity (%)	2.3 ± 0.3
Dissolved oxygen (mg/L)	1.0 ± 05
Sulfate (mg/L)	698 ± 11
Total chromium	85.4 ± 2.8
Total copper (mg/L)	NT
Nickel (mg/L)	NT

indicated the need for proper treatments before its release into the environment.

3.2. Screening for Cr(VI) resistant bacterial isolate from tannery effluent

Among the bacterial strains isolated from the effluent using dilution plate method as discussed in material method section, only one bacterium, *Planococcus* sp. TRC1, was found to be potent, resistant to Cr(VI) when screened on nutrient agar plates with various concentration (50–1,000 mg/L) of $K_2Cr_2O_7$. The minimal inhibitory concentration (MIC) obtained was at 1,000 and 500 mg/L in solid and in inorganic MSM, respectively. Gram staining characterization showed that the isolated bacterium was gram positive in nature and coccus in shape. This bacterium can produce orangish red pigment in solid media with or without Cr(VI) within 72 h incubation. It can grow in a temperature range of 4–42°C, at pH 6–8 and in >12% NaCl solution. The biochemical studies revealed it to be catalase, methyl red, citrate, and urea positive (Table 1(b)). The 16S rDNA sequence and corresponding phylogenetic tree analysis showed 96% close homology with *Planococcus maritimus* (KP8) (Fig. 1). The partial nucleotide sequence of this bacterium containing 1449bp has been deposited to EMBL and GenBank accession no. HE663167 has been assigned to this new strain [25]. Species belonging to *Planococcus* genera are generally gram positive, aerobic, cocci, able to grow at moderately low temperatures, high salt concentrations, and yellow to orangish red color pigment producing. In the present investigation, similar characteristics have been observed for the isolated strain confirming that the genus to be *Planococcus*. Predominantly, these bacterial species are isolated from cold and/or saline environments. The G + C content of bacterial strains from *Planococcus* genus was about 42% as reported in [26].

Table 1b
Morphological, physiological, biochemical, and molecular characterization of tannery effluent isolated bacterium TRC1

Morphological/physiological/ biochemical/molecular characteristics	Isolated bacterium TRC1
Gram stain	+
Cell shape	Cocci
Characteristics in agar plate	Soft, smooth
Growth at temperature (°C)	4–42
Growth at pH	3–9
Growth on NaCl (%)	12
Pigment production	Orangish red with λ_{max} 408–466
Indole	−
Methyl red (MR)	+
Voges-Proskauer (VP)	−
Citrate utilization	+
Glucose	+
Adonitol	−
Arabinose	−
Lactose	−
Sorbitol	−
Manitol	−
Rhamnose	−
Sucrose	−
Catalase	+
Urea hydrolysis	+
Hydrogen sulfide production	−
Caesin hydrolysis	+
Starch hydrolysis	−
Growth in	
Phenol (mg/L)	200
Catechol (mg/L)	90
O-cresol (mg/L)	100
Trichlorophenol (mg/L)	150
16S rDNA	*Planococcus* sp.

In this study, the isolated *Planococcus* sp. TRC1 bacterium has been found to tolerate 19.23 mM Cr(VI) concentration in MSM. The unique feature of such growth is that the bacterium grows entirely in inorganic media in the presence of minimal organic carbon source. Various bacterial species such as *Bacillus* [12], *Pseudomonas KK15* [27], and *Staphylococcus* [28] have been reported to resist Cr(VI) in organic media at 20, 15 and 0.054 mM concentration, respectively. The tolerance to Cr(VI) in inorganic media showed by *Planococcus* sp. TRC1 in this study, therefore appears significant. Chowdhury et al. [29], Nithya et al. [30], and Subramanian et al. [31] reported that in

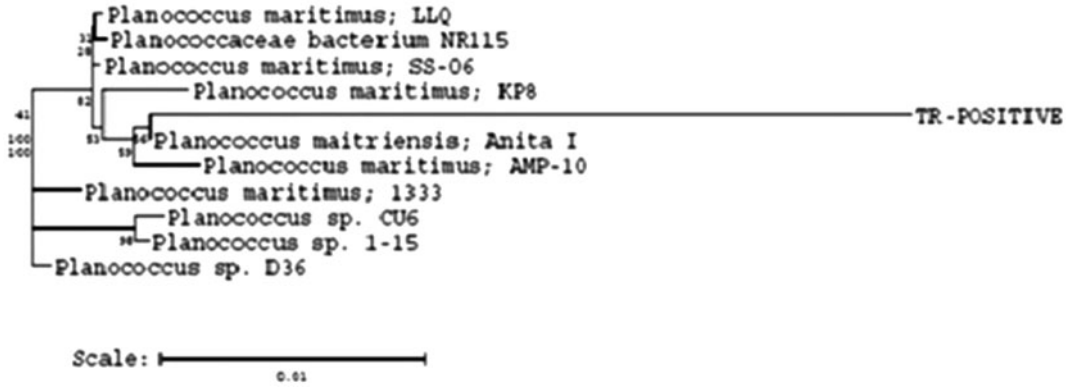

Fig. 1. 16S rDNA sequencing and phylogenetic analysis to represent close homologs of the isolated bacterial strain TRC1 from toxic tannery effluent with EMBL-bank accession number HE663167.

organic media, there were few bacterial species belonging to *Planococcus* genus which shows appreciable resistance to heavy metals. However, heavy metal tolerance in inorganic media has not been much studied on *Planococcus* sp. so far. One of the most important characteristics of this isolated strain is that it can grow in the presence of phenolic compound namely phenol, catechol, cresol, and trichlorophenol as sole source of carbon with MIC at 200, 90, 100, and 150 mg/L, respectively (Table 1(b)). The ability of the isolated strain to form the specific orangish red pigment having λ_{max} in the range 408–466 nm, when extracted using acetone to methanol in the ratio 7 to 1 (v/v) clearly indicates the presence of carotenoid group of pigments [32].

3.3. Specific growth rate of Planococcus sp. TRC1 in different concentration of Cr(VI)

The specific growth profile of the acclimatized bacterial isolate in the increasing concentration of tannery effluent and $K_2Cr_2O_7$ (2.5–500 mg/L) as source of Cr(VI) in MSM was studied at 30˚C, pH 7.2, and 120 rpm. The results (Fig. 2(a) and (b)) indicated that the isolated strain which is both Cr(VI) and phenolic component resistant can grow well in tannery effluent and in Cr(VI) containing aqueous minimal medium in concentration dependent manner. In native tannery effluent, the lag and exponential periods were found to be within the range 8–10 h and up to 34 h, respectively. On the other hand, in the presence of 25 mg/L of simulated Cr(VI) solution, the lag and exponential periods are observed to be 12–16 h and up to 42 h, respectively.

From the cell growth curve obtained at different initial Cr(VI) concentration, the specific cell growth rate was evaluated from the slope of the tangent drawn from the origin and dividing it by the initial cell concentration. When the specific cell growth rates were plotted against the initial Cr(VI) concentration, saturation point provided the maximum specific cell growth rate (μ_{max}). In the present investigation within the Cr(VI) concentration range studied, the maximum specific cell growth rate has obtained are shown in Table 2. The results clearly indicates that the isolated bacterial strain *Planococcus* sp. TRC1 can well tolerate the toxic and hazardous microenvironment of the effluent along with appreciable high Cr(VI) concentration.

3.4. Dynamics of chromium(VI) and COD reduction using Planococcus sp. TRC1

Studies on the dynamics of Cr(VI) removal from the simulated aqueous solution have been carried out to establish the Cr(VI) resistant nature of the isolated strain *Planococcus* sp. TRC1. The results are shown in Table 3. Depletion of Cr(VI) as a function of time has been plotted in Fig. 2(a) for an initial concentration of Cr(VI) of 25 mg/L. It is observed that the depletion dynamics is exponential in nature indicating very high affinity of the isolated strain towards Cr(VI) removal. Fig. 2(a) also indicates that after 48 h, the maximum removal (75 ± 2%) of Cr(VI) is achieved. The same trend was observed for the other initial concentrations of Cr(VI) up to 50 mg/L. Above this value, it is observed that *Planococcus* sp. TRC1 is not very effective in removing Cr(VI) from aqueous solution possibly due to inhibition effect of high concentration of Cr (VI) on the metabolic function of the cell. Since conventional physicochemical treatment can bring down the concentration of Cr(VI) in tannery waste effluent below 100 mg/L, the isolated strain *Planococcus* sp. TRC1 is, therefore, expected to be highly

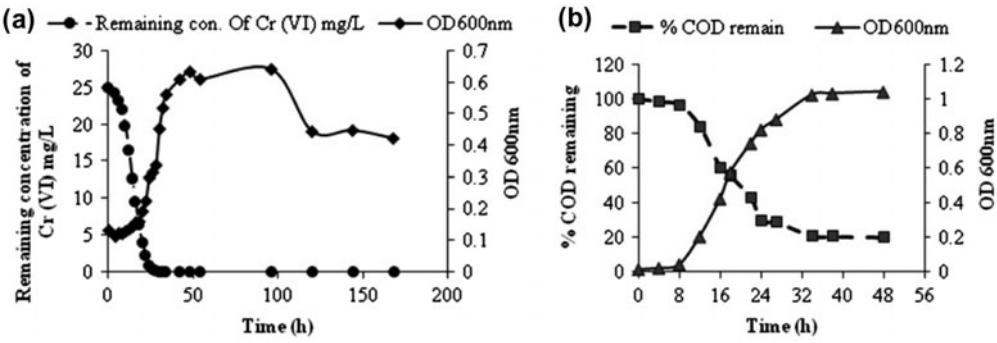

Fig. 2. Hexavalent chromium and COD reduction by *Planococcus* sp. TRC1 with time: correlation between growth of *Planococcus* sp. TRC1 and (a) reduction of Cr(VI) in aqueous solution of 25 mg/L of $K_2Cr_2O_7$ as Cr(VI) source and (b) COD reduction of raw tannery effluent at 30°C, pH 7, and 120 rpm.

Table 2
Specific growth rate (h^{-1}) of *Planococcus* sp. TRC1 in different concentration of Cr(VI)

Concentration of Cr(VI) (mg/L)	Specific growth rate (h^{-1})
2.5	0.188
5	0.197
7.5	0.210
10	0.239
25	0.276
50	0.162
100	0.084
200	0.066
300	0.054
500	0.049

effective for bioremediation of tannery waste for the purpose of removing Cr(VI) from the solution.

Presence of Cr(VI) in the tannery waste increases COD to a large extent. The removal of Cr(VI) with passage of time will simultaneously reduces the COD of the solution. In order to study the COD reduction dynamics in the presence of *Planococcus* sp. TRC1, experimental data of % COD removal against time have been plotted in Fig. 2(b). It was observed that with an initial COD value of 7,270 ± 45 mg O_2/L, the isolated strain has been able to reduce the COD value to almost nearly 20% of the initial value. This observation evidently indicates that the removal of Cr(VI) has been efficiently done by the isolated strain *Planococcus* sp. TRC1. It may therefore infer that the isolated strain *Planococcus* sp. TRC1 not only effective in inorganic media with minimal glucose concentration but the strain is also highly effective in actual tannery waste effluent containing several toxic materials other than the targeted Cr(VI).

A literature survey [33,34] shows that although several *Planococcus* strains are capable of reducing COD from industrial wastes, no reports have so far been presented on the effectiveness of *Planococcus* sp. TRC1 on the reduction of COD from tannery waste effluent containing Cr(VI). Thus, *Planococcus* sp. TRC1 isolated in the present investigation is probably the first reported micro-organism capable of reducing Cr(VI) as well as bringing down the COD level to a

Table 3
Removal of Cr(VI) from aqueous solution by *Planococcus* sp. TRC1 as a function of time

Concentration of Cr(VI) (mg/L)	% Cr(VI) removal with time (h)			
	24 h	32 h	48 h	72 h
10	46 ± 4	52 ± 3	64 ± 2.5	63 ± 1
25	62 ± 3	66 ± 4	75 ± 2	75 ± 3
50	57 ± 2	61 ± 5	65 ± 3	65 ± 2
100	15 ± 4	22 ± 2	32 ± 2	45 ± 3
200	7 ± 1	11 ± 3	13 ± 2	18 ± 2

Note: The batch study was conducted with 10% of inoculum volume at 30°C, pH 7.2, and 120 rpm for different time period. (Data represent the mean ± standard deviation of three individual experiments.)

significant level from tannery waste. It may however, be mentioned that Subramanian et al. [31] have successfully utilized *P. maritimus* VITP21 for reduction of Cr(VI) from well programed Luria broth (LB broth), but no attempt has been made by them to study the effectiveness of their cell in actual tannery waste.

3.5. Optimization of experimental conditions using one factorial method

3.5.1. Effect of initial pH, temperature, and inoculum volume on Cr(VI) removal by Planococcus sp. TRC1

For the optimization of experimental conditions in *Planococcus* sp. TRC1-mediated Cr(VI) removal study, one factorial method was chosen. Experiments were performed for 24 h at a fixed initial concentration of Cr(VI) of 25 mg/L, varying one of the experimental conditions such as pH (5–9), temperature (20–40˚C), and % inoculum (5–25), for a particular time keeping other conditions fixed. The optimum pH value was found to be 7.2 when the maximum Cr(VI) removal was obtained to be 72 ± 4%. This important observation leads to the conclusion that pH of the tannery waste effluent must be brought to nearly 7.0 in order to get the maximum activity of *Planococcus* sp. TRC1.

Experimental effect of temperature on removal of Cr(VI) indicates that at 30˚C the maximum % Cr(VI) (69 ± 3) is achieved. Temperatures higher than 30˚C reduce the activity of *Planococcus* sp. TRC1, possibly deactivating functional groups such as amino, carbonyl, sulfhydryl, and phosphate of protein and lipids of bacterial surface. Below 30˚C, evidently the barrier activation energy cannot be crossed.

In order to optimize the volume of inoculum, experimental runs were conducted by varying inoculum volume within the range of 5–15%, keeping pH of the solution (7.2) and temperature (30˚C) fixed. Results shows that with the increasing inoculum volume, the increasing % removal of Cr(VI) was achieved up to 15% inoculum volume. Above 15% inoculum volume, no significant increase in Cr(VI) removal was achieved, such behavior can be explained by noting that increasing inoculum volume increases the number of cells which in term provides enhancement in bacterial cell surface area. Moreover, more the cell number more is the availability of the functional groups to interact with the metal ions [35]. Experimental data also shows that while 15% inoculum volume gives the maximum removal of Cr(VI) (74 ± 4), the efficiency difference between 10 and 15% inoculum volume is significantly low. Thus from economic point of view, in all runs 10% inoculum volume was used in the present investigation.

3.6. SEM analysis

SEM photomicrographs of *Planococcus* sp. TRC1 grown in the presence and absence of Cr(VI) were presented in Fig. 3. These studies revealed that in the absence of Cr(VI), the bacteria had smooth surface and round shape where as in the presence of Cr(VI) it showed round globules and amorphous substances aggregated all over the cell surface of *Planococcus* sp. TRC1. The cell wall became rough and the cell changed to slightly rod shaped but there was not much change in cell surface compared to the control. The elongation and deformation of the cell may be due to the heavy metal stress that was similar to the finding of Francisco et al. [36]. Xie et al. [37] also reported similar types of micrographs in *Enterobacter cloacae* before and after CR(VI) loading.

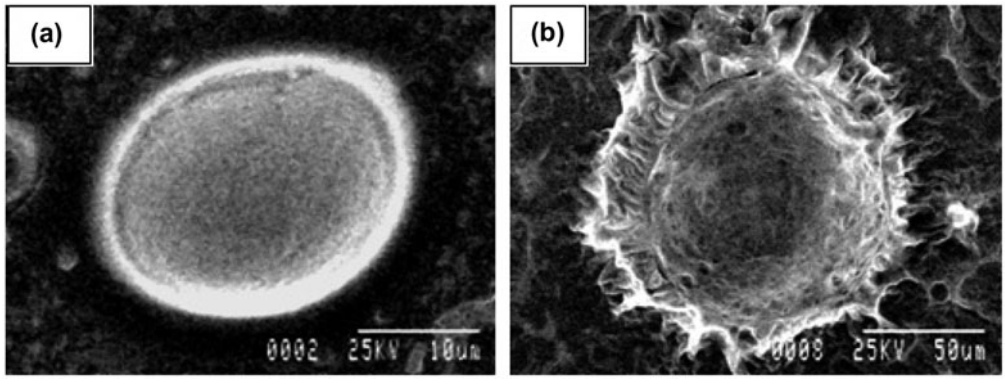

Fig. 3. Scanning electron micrograph of *Planococcus* sp. TRC1 in (a) absence and (b) presence of Cr(VI).

3.7. FTIR analysis

In order to confirm the involvement of functional groups in biosorption of Cr(VI) on the cell surface of the isolated strain *Planococcus* sp. TRC1, the Fourier transform infrared spectroscopic (FTIR) study was investigated. The FTIR spectra of *Planococcus* sp. TRC1 grown MSM in the presence and absence of 25 and 100 mg/L of Cr(VI) solution was analyzed in the range of 400–4,000 cm^{-1} using standard protocol and compared. From the results (Table 4), it is clearly evident that the spectrum of the biomass displayed a number of absorption peaks reflecting the complex nature of biomass.

Conspicuous changes in the FTIR spectrum of the isolated strain *Planococcus* sp. TRC1 was observed when grown in the absence and presence of 25 and 100 mg/L of Cr(VI). Shifting of the peak at 3,421, 1,560, and 1,458 cm^{-1} in the absence of Cr(VI) to 3,398, 1,542, 1,406 cm^{-1} and 3,368, 1,543, 1,402 cm^{-1} in the presence of 25, and 100 mg/L solution of Cr(VI), respectively, suggest that Cr(VI) metal binding process took place on the surface of the cells with the involvement of specific functional groups viz., –OH group, primary and secondary amines, amides stretching (N–H stretching), amides bending, C–N stretching, and N–H deformation. Most bacterial surface groups were either protonated and neutrally charged or deprotonated and negatively charged, although amine groups were positively charged when protonated. Aqueous metal cations interact both eletrostatically and covalently with deprotonated functional groups. The peak at 1,152 and 1,080 cm^{-1} appeared on biomass was totally masked in biomass with Cr(VI) at 100 mg/L. Absorption at 1,240 cm^{-1} remains same indicating the presence of $–SO_3$ group in both the cases. The changes in the adsorption peaks may be due to the adsorption of Cr(VI) in the cell surface. All the observed changes could be due to the oxidation of biomass during the biosorption of Cr(VI) [22]. Recently, Latha et al. [38] reported that bacterial cells in the presence of Cr(VI) secrete high molecular mass polymers that might be released to the environment or remain attached to the cell surface responsible for adsorption of metal ions to bacterial cell surface. Moreover, the cell wall components of the gram positive bacteria such as peptidoglycan, teichoic, and teichouroic acid are known for metal ion interaction [39,40].

The observed results on FTIR study indicated the maximum involvement of amide group, amide bending, –N–H stretching when the bacterial cells were grown in the presence of Cr(VI). This reflects that Cr(VI) in the form of CrO_4^{2-} oxyanions from aqueous solution interact via amine groups which are positively charged when protonated on the surface of *Planococcus* sp. TRC1 (Table 4). The batch study on Cr(VI) removal, adsorption isotherm analysis and FTIR analysis supports that *Planococcus* sp. TRC1 bacterium has the potential to remove Cr(VI) in a significant manner and the probable mechanism could be biosorption. Biosorption processes are industrially accepted process as it could not only detoxify the hazardous heavy metals from the wastewater but valuable metals could be removed and reused for the specific purpose. So this bacterial isolate could be industrially useful for heavy metal detoxification.

Table 4
FTIR spectral analysis of *Planococcus* sp. TRC1 with and without the presence of hexavalent chromium

Wave length (cm^{-1})	*Planococcus* sp. TRC1 (without Cr)	*Planococcus* sp. TRC1 (25 mg/L)	*Planococcus* sp. TRC1 (100 mg/L)	Assignment
3,500–3,200	3,421	3,398	3,368	Bonded –OH group Primary and secondary amines and amide stretching
3,000–2,850	2,926	2,926	2,926	C–H stretching
1,670–1,640	1,654	1,654	1,655	C=O stretching, amide groups
1,640–1,550	1,560	1,542	1,543	C–N stretching, N–H deformation amines, oximes
1,550–1,400	1,458	1,406	1,402	(COO^-) amino group
1,400–1,240	1,239	1,240	1,240	$–SO_3$ group
1,240–1,024	1,152	1,152	–	C–O stretch/P=O
1,100–1,000	1,079	1,079	–	C–O stretching and carboxylic acid
1,100–1,000	1,024	1,031	1,031	C–O

Note: FTIR analysis of *Planococcus* sp. TRC1 grown in the absence and presence of (25 and 100 mg/L) Cr(VI) in minimal salt media at 30°C, 120 rpm and dried at 50°C in a dry air oven and sample was prepared as mentioned in materials method.

3.8. Equilibrium modeling adsorption isotherm on the Cr (VI) removal by live Planococcus sp. TRC1 monoculture

A literature survey shows that metal pollutants can only be transformed to less-toxic oxidation states or removed either by adsorption or accumulation. Among the various approaches currently employed, biological methods for Cr(VI) detoxification seem to be the most acceptable approaches [41]. In the present investigation, SEM picture and FTIR study evidently indicates that the separation of Cr(VI) in aqueous solution is by adsorption on the outer cell surface. In order to predict the adsorption isotherm two standard adsorption isotherms namely Freundlich adsorption isotherm and Langmuir adsorption isotherm have been investigated. The quantitative equations for the two isotherms are as follows:

Freundlich isotherm

$$\log\ q_e = \log\ K_F + \frac{1}{n(\log\ C_e)} \quad (1)$$

where K_F is the Freundlich constant and n is the Freundlich exponent known as adsorbent intensity. The higher the value of K_F and n, the affinity of biomass to metal is higher [42].

Langmuir isotherm

$$\frac{1}{q_e} = \left(\frac{1}{bq_m} \times \frac{1}{C_e}\right) + \frac{1}{q_m} \quad (2)$$

where C_e is the equilibrium concentration (mg/L), q_e is the amount of Cr(VI) adsorbed at equilibrium (mg/g), and q_m (mg/g) and b (l/mg) are Langmuir constants showing the adsorption capacity and energy of adsorption, respectively.

Eq. (1) clearly indicates that the plot of $\log q_e$ vs. $\log C_e$ should be linear, if Freundlich adsorption isotherm is valid. On the other hand, Eq. (2) when rearranged indicates the plot $1/q_e$ vs. $1/C_e$ should be linear if Langmuir adsorption isotherm is followed by the system.

Fig. 4(a) shows the plot of experimental data on $\log q_e$ vs. $\log C_e$ coordinates. It is immediately evident that a good linear fit of experimental data have been obtained with R^2 value 0.987. On the other hand, when experimental data have been plotted in double reciprocal form of $1/q_e$ vs. $1/C_e$ (Fig. 4(b)) it is observed that cell surface adsorption of Cr(VI) does not follows Langmuir adsorption isotherm.

Fig. 4(a) conclusively proves that the adsorption isotherm of Cr(VI) on cell surface follows Freundlich adsorption isotherm. For the purpose of comparison of the adsorption isotherms, parameters are given in Table 5.

Applicability of Freundlich model in this study implies the involvement of heterogeneous surface condition for Cr(VI) removal by live bacterial system. Among the two distinct processes known, such as bioaccumulation and biosorption, *Planococcus* sp. TRC1-mediated Cr(VI) removal appears to be via biosorption.

3.9. Ecotoxicity test

The importance of the proper risk assessment associated with biological treatment process is a serious concern as the wastewater contains many hazardous organic chemicals and heavy metals some

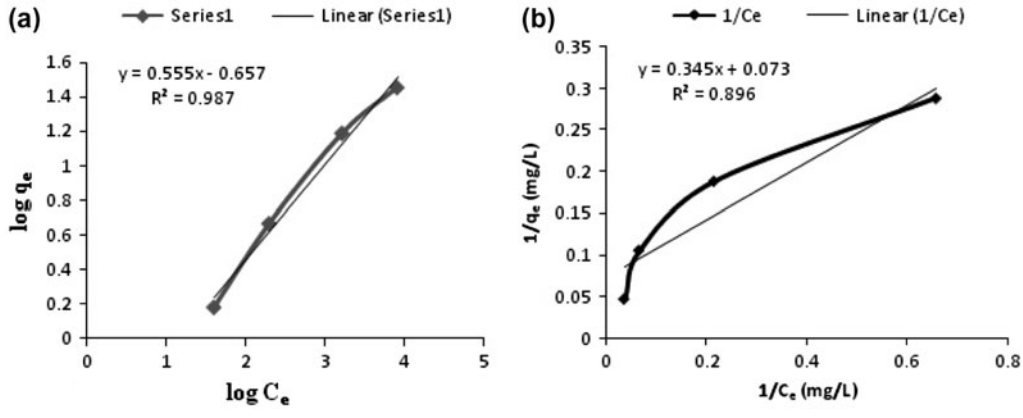

Fig. 4. Adsorption isotherm: analysis of Cr(VI) adsorption through heterogeneous surface of *Planococcus* sp. TRC1 with time (a) Freundlich plot $\log q$ vs. $\log C$ and (b) Langmuir plot $1/q_e$ vs. $1/C_e$ for various concentration of Cr(VI) at optimum experimental conditions.

Table 5
Comparison of adsorption isotherms parameters

Name of the micro-organisms	Freundlich isotherm			Langmuir isotherm			Refs.
	K_F	$1/n$	R^2	q_m	b	R^2	
Escherichia coli	0.408	0.4878	0.991	9.533	0.0077	0.943	[43]
Bacillus coagulans	0.431	0.571	0.970	19.455	0.0052	0.976	[43]
Streptococcus equisimilis	1.226	0.465	0.984	16.077	0.0020	0.931	[43]
Bacillus subtilis	2.7179	0.513	0.972	10.526	0.0874	0.997	[44]
Pseudomonas aeruginosa	15.299	0.105	0.998	3.496	0.2977	0.988	[44]
Enterobacter cloacae	366.437	0.144	0.911	25.641	0.0030	0.999	[44]
Planococcus sp. TRC1	0.2209	0.555	0.978	13.69	0.2117	0.897	Present study

of which are known as mutagenic and can harm the ecosystem. After any treatment, the byproducts generated must be evaluated with respect to the toxicity to ecosystem. To confirm the eco-friendly nature of the biotreatment of tannery effluent and aqueous Cr(VI) solution by the isolated strain *Planococcus* sp. TRC1, *A. cepa* toxicity test was investigated. The toxicity test for real tannery effluent and Cr(VI) at 25 mg/L before and after *Planococcus* sp. TRC1 treatment was examined. The results represented in Table 6 shows that the root length of *A. cepa* when exposed to the *Planococcus* sp. TRC1 treated Cr(VI) solution and tannery effluent have enhanced when compared to their respective untreated controls. About 2.5-fold root length, enhancement for bacterial-treated Cr(VI) solution (43%) compared to untreated 25 mg/L Cr(VI) as control (67%) was obtained. It was also found that for bacterial-treated tannery effluent nearly about twofold enhancement in the root growth (37%) compared to the untreated tannery effluent (62%).

The MI was found to be effected too when analyzed microscopically in *A. cepa* root meristematic cells. The % MI was calculated by dividing the number of cells in mitosis by total number of cells scored and multiplying the results by 100. The results in Table 6 clearly indicated that *Planococcus* sp. TRC1 treatment to Cr(VI) and tannery effluent-affected cell division and about twofold (40 ± 3.38) enhancement in MI compared to their respective untreated controls. It was known that with the decrease in MI, the level of cytotoxicity was increased and vice versa [45,46]. The same trend has been reflected in the present investigation that untreated tannery effluent and Cr(VI) being toxic decreased the MI when compared to normal tap water. However, *Planococcus* sp. TRC1 mediated Cr(VI) removal from aqueous solution enhanced the rate of cell division and improved root growth confirm that toxic components and heavy metals from the system have been decreased without any toxic intermediates formation during bacterial treatment with time. Literature study shows that the decrease in root length is due to heavy metal stress in trees and crops [47]. Prasad et al. [48] reported that among the heavy metals "Cr" is more toxic and affect the root growth of *Salix viminalis* than any other heavy metal. In the present investigation, the reduced genotoxicity of wastewater after the biotreatment provides the information which contributes to a proper decision for selecting the treatment strategy for the disposal of industrial effluent to receive water body. Results in this study thus suggest that *Planococcus* sp. TRC1 monoculture treatment could be an eco-friendly

Table 6
Effect of bacterial treatment on Cr(VI) and tannery wastewater induced toxicity to *Allium cepa*

Test samples	Root length (cm)	% Root length inhibition	% of Mitotic index
Negative control (tap water)	4.5 ± 0.9	0	100 ± 23
25 mg/L Cr(VI) solution	1.5 ± 1.4	67 ± 2.4	20 ± 3.22
72 h *Planococcus* sp. TRC1-treated 25 mg/L Cr(VI) solution	3.4 ± 1.2	24 ± 2.8	40 ± 3.38
Tannery wastewater (100%)	1.7 ± 0.26	62 ± 3	44 ± 17
24 h *Planococcus* sp. TRC1-treated tannery wastewater	3.36 ± 0.06	25 ± 2	87 ± 10

Note: Data represent the mean ± standard deviation of three individual experiments.

process with no toxic secondary metabolites generation and consequently no toxicity enhancement.

Further it could be said that this specific indigenous bacterial strain of *Planococcus* genus could be useful in treating leather industry effluent with respect to Cr(VI) and COD reduction both without any hazardous environmental impact.

4. Conclusion

In this study, a potent Cr(VI) and phenolic compound resistant bacterial strain isolated from tannery effluent, identified as *Planococcus* sp. TRC1 (96% homology with *P. maritimus*, KP8) showed significant potential in reducing both COD of tannery effluent ($7{,}240 \pm 45$ mg O_2/L) and Cr(VI) from aqueous inorganic media making the bacterial treatment economically useful. The SEM and FTIR study revealed that *Planococcus* sp. TRC1 mediated Cr(VI) removal was through biosorption. Reduction in toxicity associated with raw tannery effluent and Cr(VI) solution as analyzed from *Allium test* reveals the industrial and environmental acceptability of *Planococcus* sp. TRC1 (HE663167) monoculture-mediated waste treatment.

It is true that the biomass after the end of the process will contain an appreciable quantity of Cr(VI). This biomass can be ultra-centrifuged and ruptured. The intracellular product chromium reductase coming out from the cell can reduce Cr(VI) to its Cr(III) form. Thus, an eco-friendly process could be established.

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