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Cr (III) bioremoval capacities of indigenous and adapted bacterial strains from Palar river basin

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ABSTRACT

The effluents from tanning industries in and around Palar river basin are the major cause of Cr (III) pollution. Forty-five chromium (III) tolerant bacterial strains were isolated from the Palar river basin. *Bacillus subtilis* VITSCCr01 showed tolerance up to 1500 mg/l and its Cr (III) bioremoval capacity was 64%. Increasing the concentration of Cr (III) increased exopolysaccharide (EPS) production by the bacteria. FT-IR spectral studies confirmed the presence of polysaccharides in the Cr (III) treated bacteria. Adaptation of *Bacillus subtilis* VITSCCr01 with higher Cr (III) concentration improved the bioremoval capacity to 85%. SEM-EDX showed that the adapted bacteria accumulated high concentration of chromium. *Bacillus subtilis* VITSCCr01 could be used as a tool for *in situ* removal of Cr (III) especially in the tannery polluted environment.

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1. Introduction

Palar river basin of Vellore district, Tamil Nadu, India is home to many tanneries. Chromium and its compounds are major pollutants in Palar river basin of Vellore district. Tanning process, using chromium compounds is one of the most common methods for processing of hides [1]. Wastewaters from tanneries contain chromium in the form of Cr (III). Total chromium concentration in tannery effluent varies from 2500 to 8000 mg/l [2,3]. Apart from tanning, chromium is used in chrome plating, wood preserving, textile dyeing, pigmenting, chromium chemical production, pulp and paper industries and leather tanning. The wastewater resulting from these processes contain high amounts of chromium metal, which is harmful to environment and human health [4]. Palar river basin is the main drinking water source in this region. Due to mushrooming of tanneries day by day the present study becomes imperative to alleviate the problem of chromium.

In the tanning process, chromium (III) compounds are used for processing of hides [1]. About 60–70% of chromium reacts with the hides and about 30–40% of the chromium remains in the solid and liquid wastes (especially spent tanning solutions) and these are significant sources of chromium pollution to the environment. The most common species of chromium from tannery effluent is Cr (III). In the environment Cr (III) get oxidized to Cr (VI) by MnO and bacteria present in the soil [5]. Trivalent chromium helps in glucose and lipid metabolism, on the other hand it causes skin allergy with long time contact that leads to cancer [6,7]. Kusiak et al. [8] reported that Cr (III) increased mortality in miners. Trivalent chromium is toxic to fish when its concentration in water exceeds 5 mg/l [9]. Trivalent chromium species normally carry positive electric charges and therefore can be easily adsorbed on the negatively charged soil particles [10,11] and leads to pollution of soil environment thereby entering the food chain.

Conventional methods for removing metals from industrial wastewater include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery [12]. These processes are not economically viable especially when metals in solution are in the range of 1000–100 μg/ml [13]. Therefore, it is important to develop an innovative, low cost and eco-friendly method for extraction of toxic heavy metal ions present in wastewater. Microorganisms including bacteria, filamentous fungi and yeasts are found to be capable of efficiently removing heavy metals [14] including chromium [15]. In our study we have isolated Cr (III) tolerant bacterial strains from tannery polluted environment of Palar river basin, and were adapted to high chromium concentration and studied for Cr (III) bioremoval capacity. This study intends to confiscate trivalent chromium from the environment and also from the tannery effluent which is the main source of chromium pollution in Vellore District.

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2. Materials and methods

2.1. Sample collection

Soil samples were collected from the top soil horizon (0–20 cm) of the chromium polluted sites in the Palar river basin of Vellore district, Tamilnadu, India. The samples were brought to the laboratory in ice boxes and processed for the isolation of Cr (III) tolerant bacterial strains as per APHA [16].

2.2. Enrichment and isolation of Cr (III) tolerant bacterial strains

10 g soil was added into100 ml of nutrient broth containing Cr (III) in the form of chromium nitrate (the pH of the media was adjusted to 4 and maintained throughout the incubation period) and incubated at 30 °C for 4–6 days. Thereafter, the culture broth was serially diluted into series of dilution sequences used for isolation of Cr (III) tolerant bacteria in nutrient agar plates in pH 4 amended with $Cr(NO_3)_3{\cdot}9H_2O$ (0.07697 g in 100 ml of the medium which is equal to 100 mg/l of Cr (III)). The plates were incubated at 30 °C for 3–5 days.

2.3. Evaluation of tolerance to chromium and other metals

The maximum tolerable concentration (MTC) of all the Cr (III)-resistant isolates were determined by well diffusion method and broth dilution method [17] in PYG medium with Cr (III) concentrations ranging from 100 to 4000 mg/l. The maximum concentration of metal in the medium which support the growth was taken as the maximum tolerable concentration (MTC).

The isolated chromium tolerant bacterial strains were also tested for tolerance to other metals like Zn, Fe, Pb, Cd and Ni at 100 mg/l by broth dilution method [17] in PYG medium supplemented with above mentioned metals. In addition, the salt tolerance was checked with NaCl in nutrient broth by broth dilution method.

2.4. Growth pattern and Cr (III) bioremoval by the isolated bacteria

A loop full culture of 5 selected strains were inoculated into 100 ml of nutrient broth separately and incubated in an orbital shaker at 150 rpm for 24 h at a temperature of 30 °C. After that, 10 ml of the grown bacterial culture was transferred into100 ml of fresh nutrient broth at pH 4, supplemented with 0, 25, 50 and 100 mg/l Cr (III) and incubated at 30 °C in shaker at 150 rpm for 72 h.

Samples were centrifuged at 10,000 × *g* and residual Cr (III) in the medium was determined from the supernatant. In order to determine the adsorbed Cr (III) on the cell surface, the pellets were further washed with 1 ml of 10 mM EDTA solution for desorption of Cr (III) from the cell surfaces and centrifuged at 10,000 × *g* once again. The pellets were re-suspended and sonicated by using an ultrasonic cell crusher (SONICS, USA) at 50 MHz on ice in 1 ml of 1N HNO_3 and the amount of intracellular accumulation of Cr (III) was determined [18].

Atomic absorption spectroscopy (VARIAN SPECTRAA 240) was used for chromium analysis. Cr (III) removal was analysed by determining the Cr (III) in the medium, i.e., Cr (III) adsorbed on the surfaces of the cells and Cr (III) accumulated in the cells. The chromium removal rate (%) was calculated using the equation given below:

$$\text{The chromium removal rate} = \left(\frac{\text{amount of removed Cr}}{\text{amount of initial Cr}}\right) \times 100$$

One portion of the supernatant from 100 mg/l amended Cr (III) samples were used to evaluate cell growth by measuring optical density at 600 nm. The control growth study was done for each bacterial strain without chromium in the nutrient medium. All the experiments were performed in triplicates.

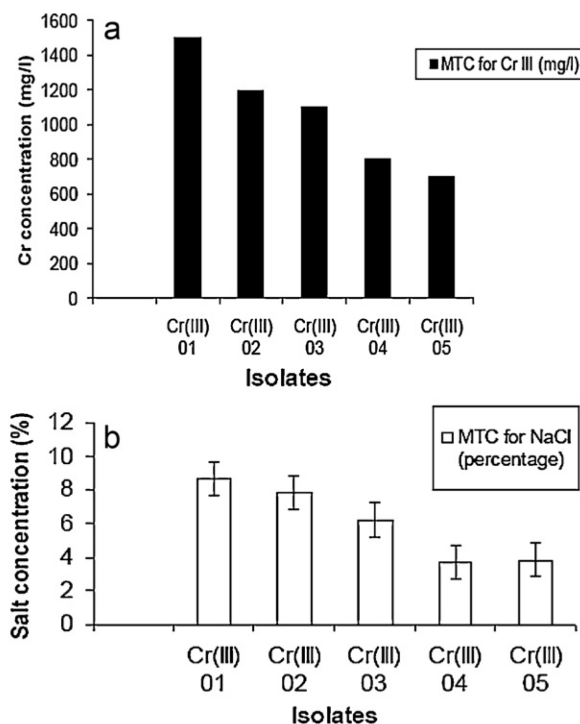

Fig. 1. (a) Maximum tolerance concentration (MTC) of the isolates for chromium (III) at 30 °C. (b) Maximum tolerance concentration (MTC) of the isolates for NaCl at 30 °C.

2.5. Extraction and quantification of exopolysaccharide (EPS)

A loop full of bacterial culture was inoculated into 50 ml of NB broth, and it was allowed to grow for 48 h at 30 °C, shaking at 180 rpm. The same experiment was carried out in NB broth dosed with 100 mg/l of Cr (III). The cultures were centrifuged at 10,000 × *g* for 10 min and the pellets were collected. It was dissolved in 1 ml of deionized distilled water and boiled for 15 min at 100 °C. It was then kept at room temperature for 10 min and 3 μl of 85% trichloroacetic acid solution (TCA) was added. The mixture was centrifuged at 10,000 × *g* for 30 min and the supernatant which contained exopolysaccharides was pooled and mixed with equal volume of ethanol. The mixture was kept at 4 °C overnight and centrifuged at 10,000 × *g* for 30 min. The precipitate was then washed two times using 95% ethanol and centrifuged at 10,000 × *g* for 30 min. Final precipitate was dissolved in 1 ml of deionized distilled water and stored at −20 °C [19]. Total exopolysaccharide was estimated in each sample by phenol–sulphuric acid method [20]. The mean values were calculated from the data obtained with triplicate trials.

Table 1
Resistance of selected bacterial isolates to Cd, Ni, Pb, Fe and Zn.

Isolates	Resistance to other metals at 100 mg/l				
	Cd	Ni	Pb	Fe	Zn
Cr (III) 01	R	R	R	R	R
Cr (III) 02	S	R	S	R	R
Cr (III) 03	R	R	R	R	S
Cr (III) 04	S	S	S	S	R
Cr (III) 05	S	R	S	R	R

R: resistant, S: sensitive.

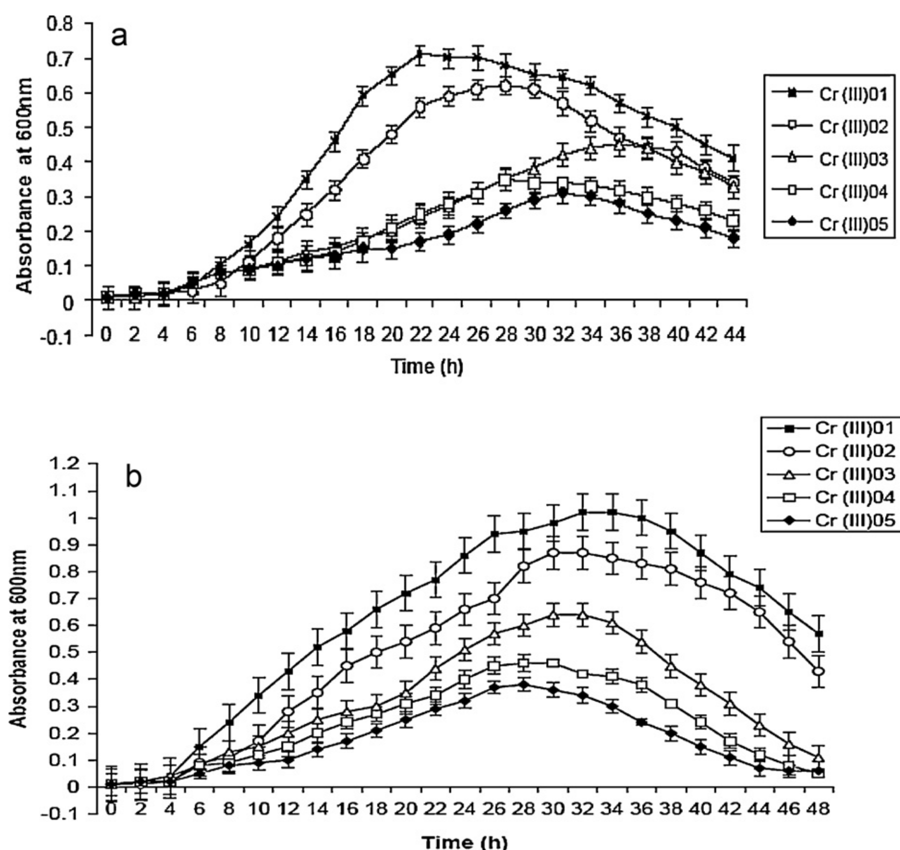

Fig. 2. (a) Growth profile of selected isolates in nutrient broth dosed with 100 mg/l Cr (III) at 30 °C under pH 3.8. (b) Control growth profile of selected isolates in nutrient broth without Cr (III) at 30 °C under pH 3.8.

2.6. Selection and characterization of effective isolate

Five morphologically distinct bacterial isolates were screened for the effective Cr (III) bioaccumulating species based on their tolerance and bioaccumulation capability. The bacterial strain with the strong ability to accumulate Cr (III) was selected for identification and was characterized morphologically, biochemically and physiologically following Gerhardt et al., [21]. The taxonomic identity of the strain was confirmed by 16S rRNA gene sequencing.

2.7. Adaptation of selected isolate

Adaptation process was performed after the enrichment, isolation and bioaccumulation studies. Adaptation was carried out by repeatedly growing the bacteria in the presence of a known concentration of Cr (III). The selected isolate was grown on NB media (pH 4) containing 1500, 2000, 2500 and 3000 mg/l chromium at 0%, 5%, 10% and 15% (w/v) NaCl concentrations on a rotary shaker at 100 rpm for 7 days at 27 ± 2 °C. In this adaptation experiment, cultures adapted to low Cr (III) and NaCl concentrations were used as an inoculum for high Cr (III) and NaCl supplemented media [22]. The experiments were repeated up to the maximum level of Cr (III) and NaCl concentrations in which the bacterial strains were able to grow.

2.8. Bioremoval of trivalent chromium by adapted bacteria

The adapted bacterial culture was again studied for its bioaccumulation capacity and its growth pattern as per method quoted in 2.4 [18] to check the difference and the effectiveness of adaptation procedure. The EPS production ability of the adapted isolate was studied at different concentrations of Cr (III).

2.9. SEM-EDX study of indigenous and adapted bacteria

Glutaraldehyde fixed and ethanol dehydrated indigenous and adapted bacteria were attached to 10 mm metal mounts using carbon tape and sputter coated with gold under vacuum in an argon atmosphere. The surface morphology of the coated samples was visualized by a scanning electron microscope (Hitachi S4000) with combined energy dispersive X-ray analyser at a voltage of ~10 keV.

2.10. FT-IR analysis

In order to investigate the functional groups involved in bioremoval of chromium in the isolate, FT-IR analysis was carried out. Infrared spectra of bacteria amended with chromium and without chromium (control) were studied. The lyophilized cell pellets were grounded and desorbed at 60 °C for 24 h and pressed to obtain IR-transparent pellets. The samples were dried and mixed with KBr (1:20; 0.02 g of sample with KBr at a final weight of 0.4 g). Infrared spectra were obtained using a Fourier transform infrared spectrometer (FT/IR-AVATAR 330). The spectra were collected within a scanning range of 400–4000/cm.

2.11. Statistical analysis

Each set of experiments was carried out at least in duplicate, and in triplicate in some cases. Experiments were repeated separately to ensure reproducibility. In each set of repeated experiments, standard deviations and standard error showed 95% confidence interval.

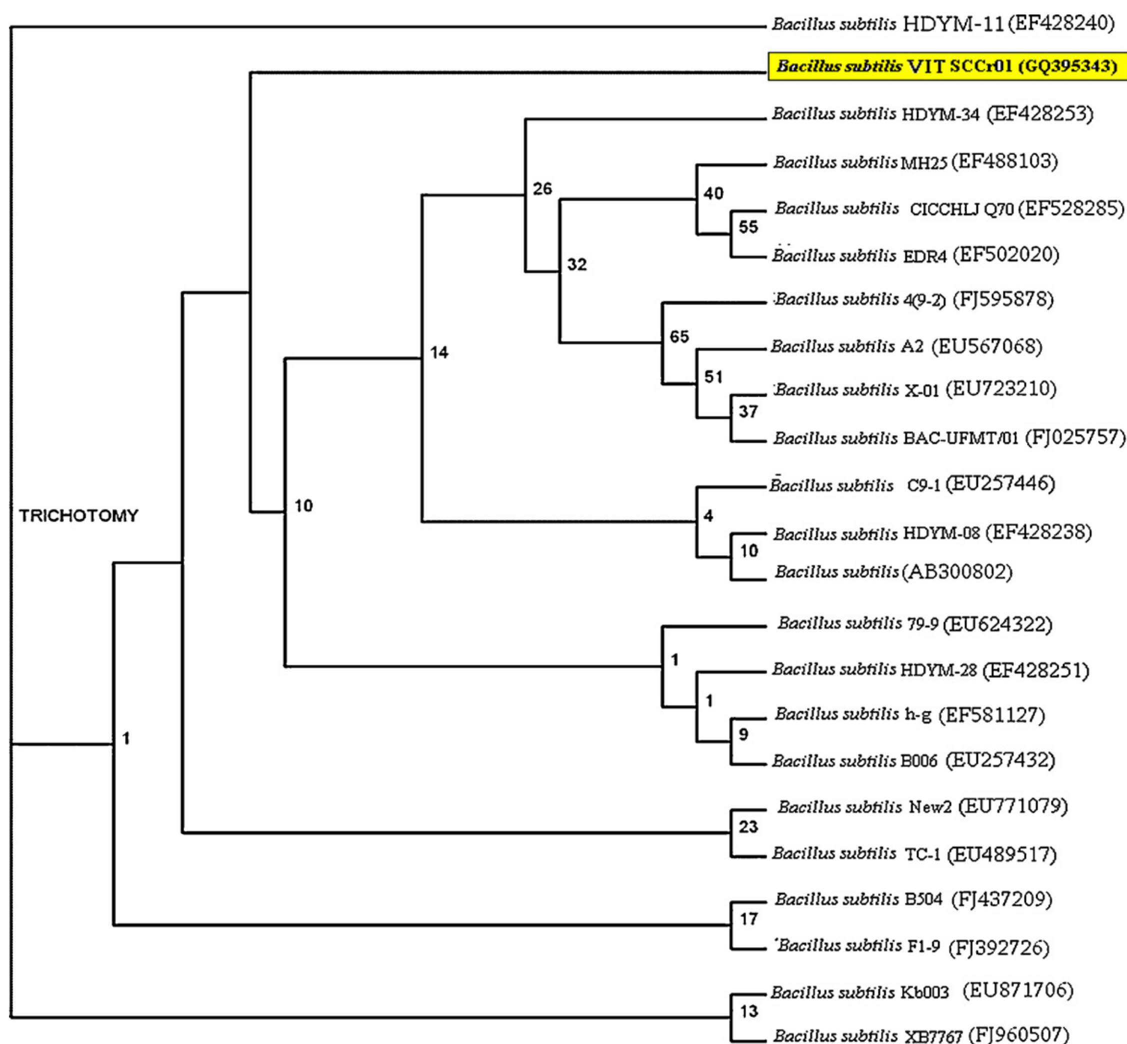

Fig. 3. The phylogram showing the position of strain Cr (III) 01 with other *Bacillus subtilis* based on 16S rRNA gene sequence. Phylogenetic tree based on neighbor joining analysis of 1000 resampled data. Number at nodes indicates the percent level of bootstrap support. Score bar represents one nucleotide substitution per 100 nucleotides.

3. Results and discussion

3.1. Isolation of Cr (III) tolerant bacteria

Forty five Cr (III) resistant bacteria were isolated from soil samples collected from Palar river basin, following dilution and plating on nutrient media amended with 100 mg/l Cr (III) in the form of chromium nitrate ($Cr(NO_3)_3{\cdot}9H_2O$). All the experiments with Cr (III) were done in acidic pH (pH 4), because Cr (III) is stable in acidic condition [23]. Isolation of bacteria from tannery environments represents an appropriate practice to select metal resistant strains that could be used for heavy metal removal and for bioremediation [24]. Chromium-resistant bacteria capable of reducing chromate have been reported from chromium polluted environments [25].

3.2. MTC (maximum tolerance concentration) of isolates to chromium (III) and salt

Tolerance to Cr (III) by the isolates was tested based on their growth in nutrient medium amended with various concentration of Cr (III) ranging from 100 to 2000 mg/l. All 46 isolates showed MTC value of 100 to 1500 mg/1 for Cr (III). Out of 45 isolates, five showed tolerance between 500 and 1500 mg/l of Cr (III) (Fig. 1a), and these were subjected to further screening. *Bacillus subtilis* VITSCCr01 (isolate number Cr (III) 01) showed a tolerance up to 1500 mg/l. The selected isolates were subjected for salt (NaCl) tolerance study and it was found that the salt tolerance capability of these isolates ranged from 4% to 9% (Fig. 1b). The isolate *Bacillus subtilis* VITSCCr01 showed maximum tolerance to NaCl, which was about 8.67% ± 0.33 (mean ± SE). Chromium (III) is not stable in neutral pH and its stability is achieved only in acidic pH [24]. Most of the studies have been done with yeast and fungi, as they can grow well in acidic pH, and withstand elevated concentration of Cr (III) [14]. There are least reports on bacterial species growing in higher Cr (III) concentration in neutral pH condition, which is required for their optimal growth. Our isolates showed resistance to other heavy metals such as cadmium, nickel, lead, and iron (Table 1).

3.3. Bioaccumulation of Cr (III) and growth pattern by the selected bacteria

The growth profiles of the 5 isolates were recorded in the presence of 100 mg/l Cr (III). Among these *Bacillus subtilis* VITSCCr01 had faster growth rate compared to others (Fig. 2a). Controls were used for all the isolates (Fig. 2b). Chromium (III) had an inhibitory effect on the growth of bacterial cells. The growth profile of the bacteria is directly related to cell density. The growth profile of the isolate *Bacillus subtilis* VITSCCr01 started its exponential phase at 6 h in control broth, but in Cr (III) amended medium, it was from 8 h. This time gap between the control and the selected strain (*Bacillus*

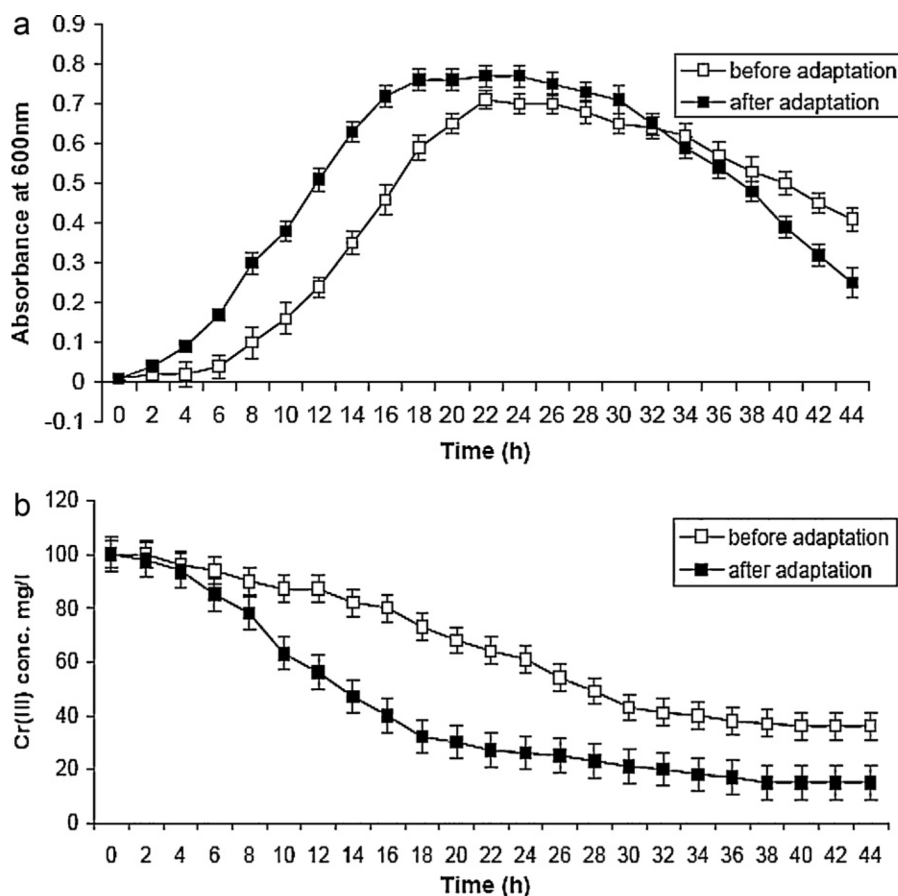

Fig. 4. (a) Growth profile of *Bacillus subtilis* strain VITSCCr01 in nutrient broth dosed with 100 mg/l of Cr (III) before and after adaptation at 30 °C under pH 3.8. (b) Bioremoval of Cr (III) by *Bacillus subtilis* strain VITSCCr01 before and after adaptation in nutrient broth dosed with 100 mg/l of Cr (III) at 30 °C under pH 3.8.

subtilis VITSCCr01) might be due to the time required for the strain to get adapted to Cr (III). The absorbance values were very less in the presence of Cr (III) compared with control. This indicated that the cell density was very less compared to control. Similar inhibitory effect was observed in other bacterial strains as well.

The bioremoval abilities of bacterial isolates were periodically monitored up to 72 h at different concentrations of Cr (III) ranging from 25 to 100 mg/l under aerobic condition at pH 3.8. At 25 mg/l Cr (III) concentration, the strain *Bacillus subtilis* VITSCCr01 (Cr (III) 01) showed 100% chromium removal within 12 h. At 50 mg/l concentration the organism took 24 h to remove 100% Cr (III). This might be due to the decrease in the uptake ability of the organism with corresponding increase in chromium concentration. The bioremoval of Cr (III) represents the cell intake and adsorption. The cell intake and adsorption of Cr (III) increased with the increase of chromium dosage in the growth medium from 25 to 100 mg/l. Chromium intake by cells was more compared to adsorption. Cell intake plays a major role in Cr (III) bioremoval. When the growth medium was supplemented with 25 and 50 mg/l of Cr (III), 100% Cr (III) removal was observed. Nearly 64% Cr (III) removal was observed at 100 mg/l concentration. The results indicated that the strain *Bacillus subtilis* VITSCCr01 has strong capacity for Cr (III) removal compared to other isolates. It was observed that pH 3.8 was the optimum pH for the growth and bioremoval of Cr (III).

The biosorption of Cr (III) was studied by Prigione et al., [14] for 5 different fungal species. The maximum Cr (III) removal was 38% from real tanning effluents. Bioremoval of Cr (III) in yeast [26,27], and lichens (*Parmelina tiliaceae*) [28] showed 29.3% and 95% Cr (III) removal respectively. Our results showed 100% bioremoval of Cr (III) by adapting indigenous bacterial species of Palar river basin.

3.4. Screening and characterization of the effective isolate (Bacillus subtilis VITSCCr01)

The isolate which showed maximum tolerance and maximum bioremoval of chromium (III) was Cr (III) 01, for which biochemical and molecular characterizations were done and identified as *Bacillus subtilis* (showing 99% similarity in BLAST search to *Bacillus subtilis*). The sequence size was 1413 bp. The phylogenetic tree of *Bacillus subtilis* strain VITSCCr01 (GenBank Accession number GQ395343) is shown in Fig. 3.

3.5. Bioremoval of Cr (III) and growth pattern by adapted Bacillus subtilis VITSCCr01

The organism which showed higher Cr (III) tolerance capacity was used for the adaptation study. To enhance the bacterial tolerance capacity to chromium and NaCl, *Bacillus subtilis* VITSCCr01 cells were adapted in nutrient broth media supplemented with incremental concentrations of Cr (III) from 250, 500, 750, 1000, 1500, 2000, 25,000, and 3000 mg/l and 0%, 2%, 4%, 6%, 8% and 10% for NaCl. It was found that the *Bacillus subtilis* VITSCCr01 microbial cells were able to survive up to 3000 mg/l chromium concentration and 15% NaCl concentrations. The growth of the *Bacillus subtilis* VITSCCr01 was studied after adaptation and observed that there was a significant increase in growth rate and bioremoval of Cr (III) (Fig. 4a and b). Before adaptation the bacteria took 6 h to start its log phase. After adaptation the lag phase of the organism started from 2 h. Fig. 4b represents the Cr (III) removal rate at each interval (2 h) from the growth medium which was supplemented with Cr (III).

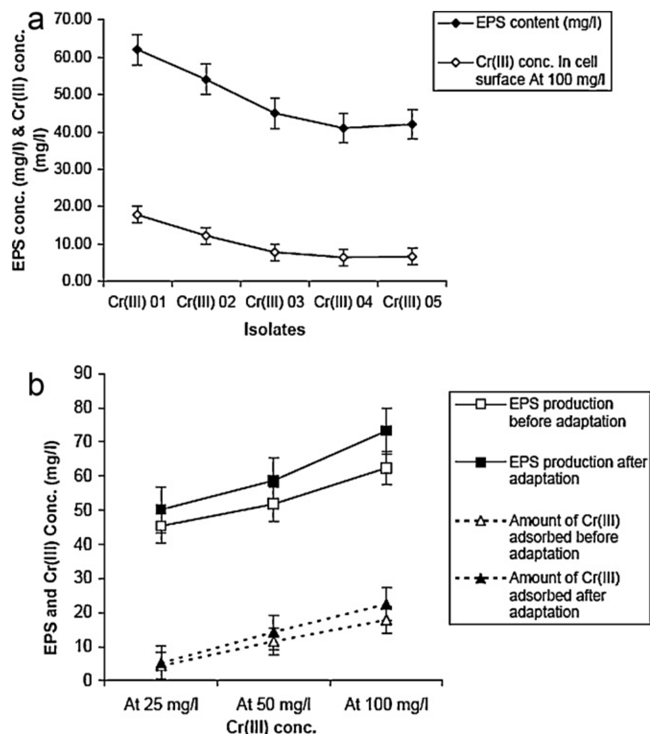

Fig. 5. (a) EPS production and Cr (III) adsorption by the selected isolates at 100 mg/l of Cr (III). (b) EPS production and Cr (III) adsorption by *Bacillus subtilis* strain VITSCCr01 before and after adaptation at different concentrations of Cr (III).

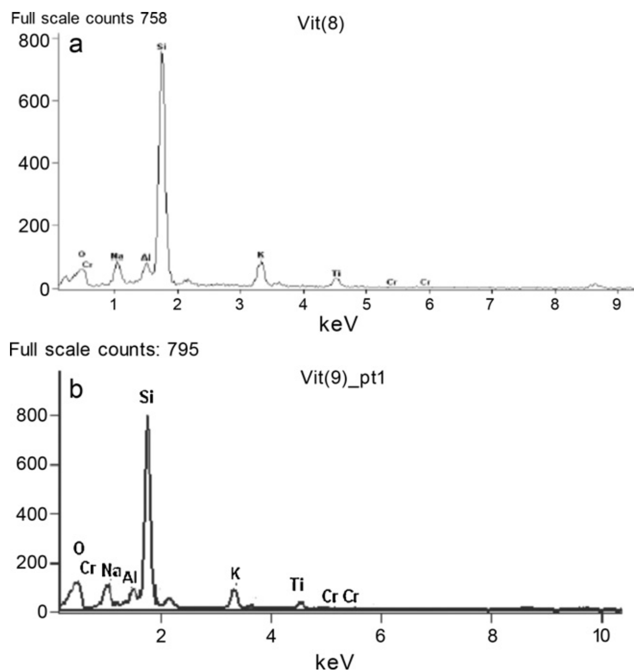

Fig. 7. (a) EDX spectra of *Bacillus subtilis* VITSCCr01 before adaptation, Si peak due to glass slide and Al peak originates from sample holder. (b) EDX spectra of *Bacillus subtilis* VITSCCr01 after adaptation, Si peak due to glass slide and Al peak originates from sample holder.

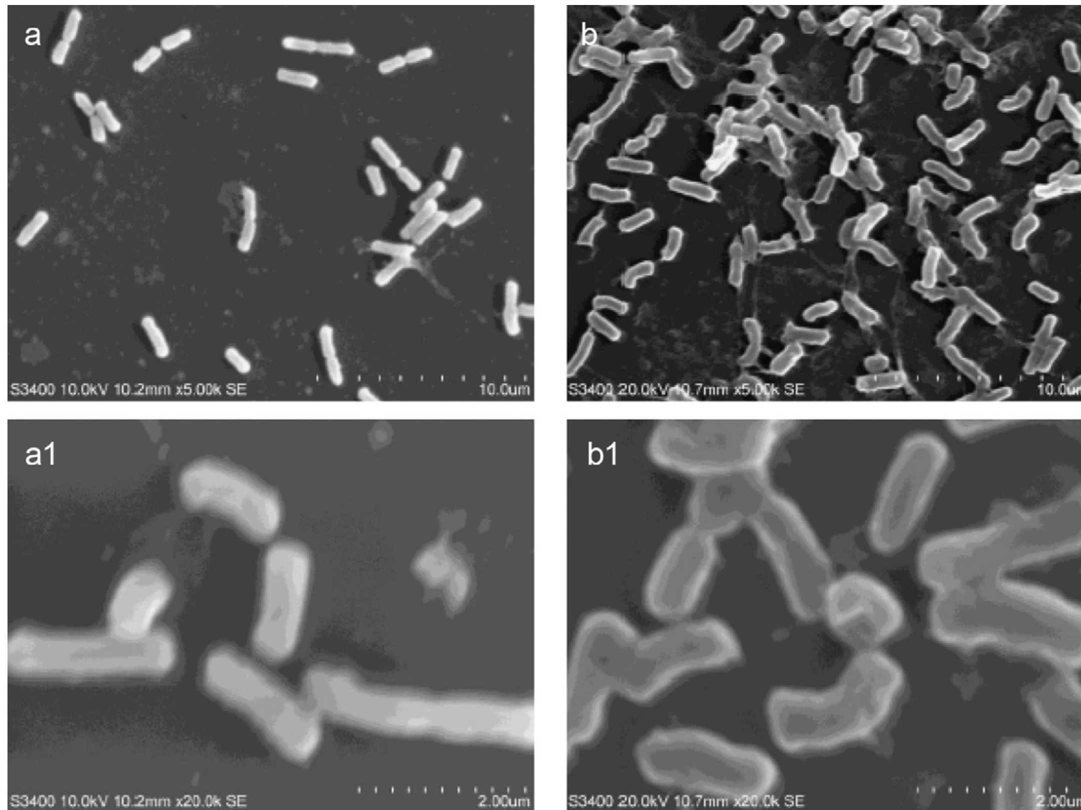

Fig. 6. (a) SEM imaging of *Bacillus subtilis* VITSCCr01 before the adaptation process showing rod shaped cells (magnification-5000×). (a1) SEM imaging of *Bacillus subtilis* VITSCCr01 before the adaptation process showing rod shaped cells (magnification-20,000×). (b) SEM imaging of *Bacillus subtilis* VITSCCr01 after the adaptation process showing increased size and altered cell morphology (magnification-5000×). (b1) SEM imaging of *Bacillus subtilis* VITSCCr01 after the adaptation process showing increased size and altered cell morphology (magnification-20,000×).

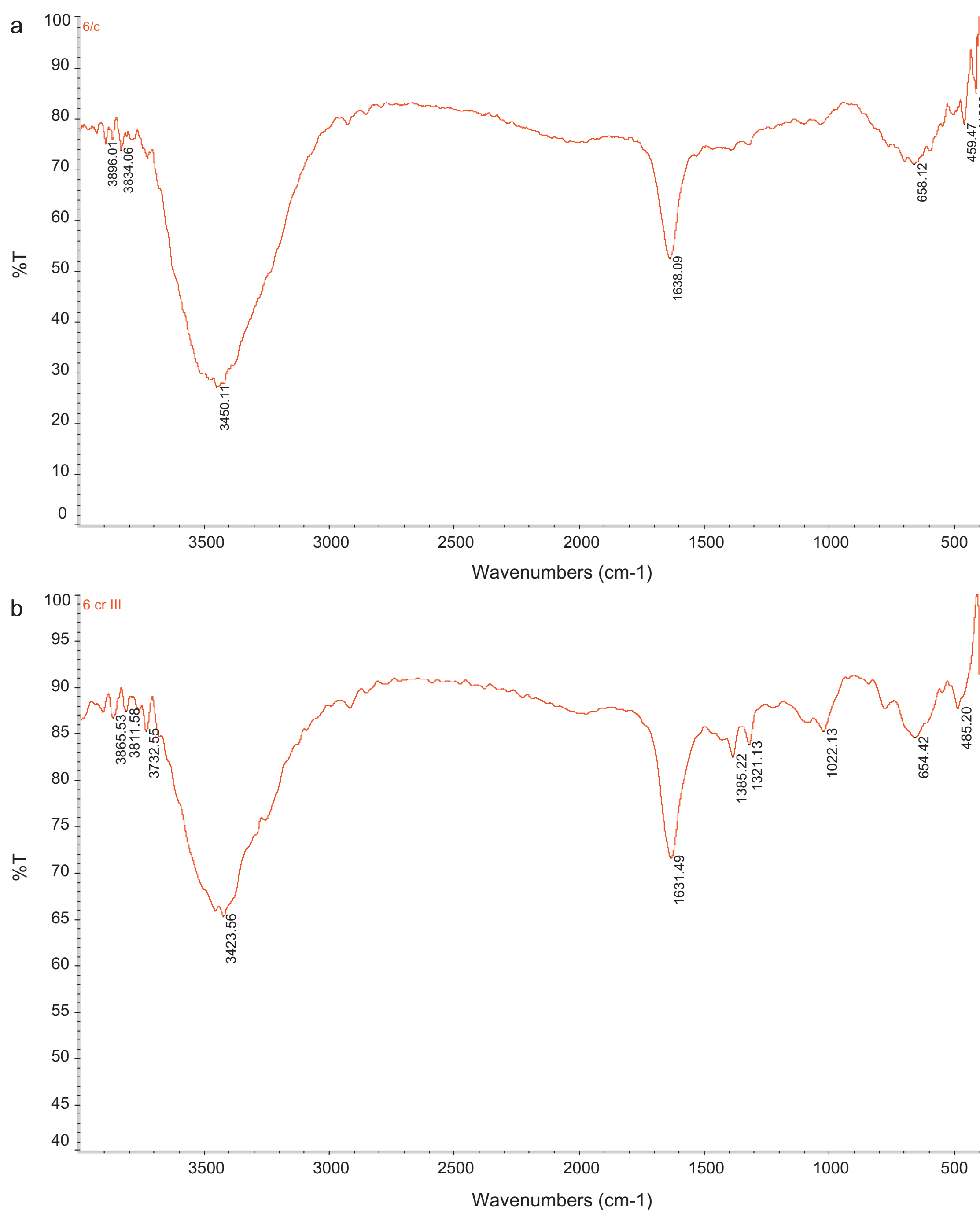

Fig. 8. (a) Fourier transform infrared absorption spectrum of *Bacillus subtilis* VITSCCr01 without chromium (III) treatment. (b) Fourier transform infrared absorption spectrum of *Bacillus subtilis* VITSCCr01 with 100 mg/l chromium (III) treatment.

The bioremoval capacity of *Bacillus subtilis* VITSCCr01 increased up to 85% after adaptation whereas, it was about 64% before adaptation.

The isolate *Bacillus subtilis* VITSCCr01 showed salt tolerance up to 9% before adaptation and it increased to 15%, after adaptation. This study parallels the finding of Dubois et al., [20] that adaptation is an effective method to increase the bioaccumulation capacity of the indigenous bacteria. A prolonged exposure to heavy metals exerts a selective pressure to the microbial community, which thereby develop resistance [27]. Our findings indicate that the adaptation might be a common phenomenon of environmental isolates that could occur when the environment is contaminated with higher concentration of pollutants like Cr (III).

3.6. Exopolysaccharide (EPS) quantification

The isolate *Bacillus subtilis* VITSCCr01 produced EPS concentration of 62.33 ± 0.33 mg/l, when it was supplemented with 100 mg/l Cr (III), than the other isolates. The EPS may play a role in the adsorption of Cr (III) in all the isolates. The greater production of EPS was observed in *Bacillus subtilis* VITSCCr01 (Fig. 5a). The production of EPS was directly related to the Cr (III) adsorption. This might be due to the secretion of high molecular mass polymers, which can either be released into the environment (EPS) or remain attached to cell surfaces [28]. The monomers of polysaccharide present in the EPS may influence heavy metal removal characteristics of these microorganisms [18].

Table 2
Percentage of elements present in *Bacillus subtilis* VITSCCr01 before adaptation through SEM-EDX analysis.

Element line	Net counts	Net counts error	Weight %	Atom %	Formula
O K	225	±40	6.83	14.84	O
Na K	948	±34	19.1	28.9	Na
Al K	920	±34	8	10.31	Al
Si K	8990	±100	–	–	
K K	1097	±38	7	6.23	K
K L	425	±21	–	–	
Ti K	375	±25	3.33	2.42	Ti
Ti L	719	±33	–	–	
Cr K	13	±11	–	–	
Cr L	318	±39	55.74	37.29	Cr
Total			100	100	

The EPS production of *Bacillus subtilis* VITSCCr01 was done before and after adaptation. There was significant increase in the EPS production after adaptation and also with increasing Cr (III) concentration (Fig. 5b). In the present study, correlation between EPS production and Cr (III) removal was analysed and it showed EPS plays a positive role in chromium bioremoval. There was an increase in the EPS production of *Bacillus subtilis* VITSCCr01 after adaptation (62.33 to 74.16 ± 0.16 mg/l). This could be due to adaptation that improved the bioaccumulation capacity of *Bacillus subtilis* VITSCCr01.

3.7. SEM-EDX study of *Bacillus subtilis* VITSCCr01

SEM imaging proved that there was a morphological discrepancy in the cells of *Bacillus subtilis* VITSCCr01 after adaptation to high chromium concentration which includes increase in the size, modification in the cell shape and cell surface alterations (Fig. 6a and b) showing the impact of chromium on *Bacillus subtilis* VITSCCr01.

SEM-EDX of adapted culture of *Bacillus subtilis* VITSCCr01 showed higher percentage of chromium. Chromium weight % was 55.74 and atom % was 37.29 before adaptation, after adaptation weight % increased to 93.37% and atom % increased to 92.22% (Tables 2 and 3). The EDX spectra (Fig. 7a and b) of both *Bacillus subtilis* VITSCCr01 and *Bacillus cereus* VITSCCr02 showed Si peak that was due the smear preparation on glass slides.

3.8. FT-IR spectroscopic analysis

FTIR spectra of chromium interacted and uninteracted *Bacillus subtilis* cells are shown in Fig. 8a and b. The spectra revealed the following bands: 3423.56 cm^{-1} for chromium interacted cells which correspond to CH_2 symmetric stretch, that could be from lipids, with little contribution from proteins, carbohydrates and nucleic acids. The spectral band at 1638 cm^{-1} correspond to C=O shifted to amide I band at 1631.49 cm^{-1} in the treated cells. The band at 1385.22 cm^{-1} and 1321.13 cm^{-1} corresponding to COO^- symmetric stretch of amino acid side chains and PO_2 asymmetric stretching which could be from nucleic acids, was more pronounced in the chromium treated cells. The peaks assigned for OH stretching of carbohydrates shifted from 3450.11 cm^{-1} to 3423.56 cm^{-1} in chromium treated cells.

Table 3
Percentage of elements present in *Bacillus subtilis* VITSCCr01 after adaptation through SEM-EDX analysis.

Element line	Net counts	Net counts error	Weight %	Atom %	Formula
Na K	609	±27	1.58	26.01	Na
Al K	721	±30	6.84	10.4	Al
Si K	6946	±87	–	–	
K K	823	±34	5.56	5.83	K
K L	910	±31	–	–	
Ti K	253	±22	2.31	1.97	Ti
Ti L	340	±26	–	–	
Cr K	10	±11	–	–	
Cr L	3597	±30	93.37	92.22	Cr
Total			100	100	

4. Conclusion

A number of technologies have been developed to remove chromium from the effluents but none seems to be effective at the industrial scale [29,30]. The present study is an effort in this direction, where Cr (III) can be removed from tanning effluent by the use of Cr (III) tolerant bacteria isolated from chromium contaminated sites. Production of exopolysaccharides is the one of the mechanism used by bacteria to remove the Cr (III) through adsorption. But the major portion of the Cr (III) was taken inside the cells. The adaptation studies proved that the bacteria can tolerate high Cr (III) concentration in the environment, and it might be helpful for Cr (III) removal. As these isolates are indigenous for these places it can be used for the *in situ* bioremediation of chromium in soil, water of Palar river basin.

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