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Hexavalent Chromium Reduction in Tannery Effluent by Bacterial Species Isolated from Tannery Effluent Contaminated Soil

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ABSTRACT

This study was conducted to isolate and characterize Cr(VI) [chromate] reducing bacteria from soil contaminated with tannery effluent (Kanpur, India) and evaluate these bacteria for Cr(VI) reduction activity. A comparative growth and reduction study was conducted in AMM (acetate minimal media; considering acetate as an economical and easily available carbon source) and in the tannery effluent for further possible application to remove Cr(VI) from the tannery effluent and other contaminated environment. The promising results encourages for the development of cost effective and user friendly bioremediation technology for tannery industry. Three bacteria (B2, B4 and B9) possessing ability to grow and reduce 1.24 mg L^{-1} of Cr(VI) below the detection limit within 24 h in absolute tannery effluent without any amendment, were evaluated for their reduction property in LB (Luria Bertani) broth media amended with 50, 100 and 200 mg L^{-1} of Cr(VI) concentrations. All three bacteria were gram positive and belong to the genus *Bacillus*. In a comparative Cr(VI) reduction study in three different media (LB, AMM and tannery effluent), isolate B9 kept the activity non-significantly different, irrespective of the type of media. However, isolate B4 showed significant influence of the media on its reduction ability. The independent growth study of these isolates distinctly indicates that tannery effluent is preferred over AMM; however, activity and growth were not linked to these isolates. The subsequent time course study further reveals their relevance and potential for application in environmental samples.

Key words: Tannery effluent, 16S rRNA identification, chromium, reduction, *Bacillus*, acetate

INTRODUCTION

Chromium (Cr) and its compounds have multifarious industrial use besides application in metallurgy they are extensively employed in finishing and processing of leather, producing refractory steel, drilling muds, electroplating cleaning agents, manufacturing catalysts and production of chromic acid and specialty chemicals. These anthropogenic activities lead to widespread chromium contamination in the environment, particularly soil and water (Azmat and Khanum, 2005; Singh *et al*., 2011; Achal *et al*., 2011).

The widespread use of chromium compounds, accounting for 40% of the total industrial use by tannery industry for tanning process is a major cause for high influx of chromium to the biosphere (Barnhart, 1997). According to the guidelines for drinking water issued by World Health Organization (WHO), the maximum permissible limit for hexavalent chromium (chromate) and total chromium are 0.05 and 2 mg L^{-1}, respectively (Gupta and Rastogi, 2009). In nature, Cr exists in two stable forms Cr(VI) and Cr (III) which are inter convertible depending on pH, inorganic ions

and organic matter content (Avudainayagam *et al*., 2006; Apte *et al*., 2005). Characteristics like higher solubility in water, rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acid makes hexavalent form of Cr comparatively more toxic than trivalent one (Basu *et al*., 1997). However, at high temperature and oxygen or through interaction with manganese dioxide (MnO_2) the trivalent Cr can be converted into hexavalent form (Apte *et al*., 2005).

Conventional treatment methodologies for soil and groundwater contaminated with Cr(VI) are based on excavation and pumping of contaminated material, followed by application of chemical reductants, which results in the precipitation and sedimentation of reduced chromium (Barakat, 2008). These processes are expensive physico-chemical methods. Biological strategies plays vital role for soil and groundwater treatment as it has potential to provide technology for microbial reduction of toxic Cr(VI) (Ganguli and Tripathi, 2002; Nasseri *et al*., 2002; Tripathi and Garg, 2010). The eco-friendly and cost-effective bioreduction of Cr(VI) is better option for treatment because of its practical importance.

Bioreduction of Cr(VI) can occur directly as a result of microbial metabolism (enzymatic) or indirectly, when mediated by bacterial metabolite (such as H_2S) (Sultan and Hasnain, 2005). A number of Cr(VI) reducing micro-organisms have been reported (Iftikhar *et al*., 2007), including *Escherichia* (Shen and Wang, 1994; Wang and Shen, 1997), *Pseudomonas* (Ishibashi *et al*., 1990; Wang and Xiao, 1995), *Bacillus* (Wang and Xiao, 1995; Garbisu *et al*., 1998; Philip *et al*., 1998; Faisal and Shahida, 2003), *Enterobacter* (Wang and Shen, 1990; Clark, 1994; Rege *et al*., 1997), *Desulfovibrio* (Lovley and Phillips, 1994), *Shewanella* (Myers *et al*., 2000), *Serratia* (Mondaca *et al*., 2002), *Rhodobacter* (Nepple *et al*., 2002) and *Arthrobacter* (Asatiani *et al*., 2004). However, the potential for the biological treatment of Cr(VI) contaminated waste is limited because some micro-organisms lose viability in high concentrations of chromate or require continuous supply of expensive nutrients for the desired activity (Gadd and White, 1993; White and Gadd, 1995; Al-Saraj *et al*., 1999; Pattanapipitpaisal *et al*., 2001; Ganguli and Tripathi, 2002; Park *et al*., 2005).

Isolating chromate reducing bacteria from contaminated soil, that could grow and reduce chromate in the contaminated environment without any amendment, could, therefore, be useful. The purpose of this study was to isolate Cr-resistant bacteria from the tannery-effluent-affected soil and select and identify those bacteria having ability to grow and reduce chromate in absolute tannery effluent, without any nutritional amendment. Extending the scope, comparative evaluation of selected bacteria for chromate reduction in tannery effluent and acetate minimal media (AMM; considering acetate as an economical and widely available carbon source) was done to assess the efficacy.

MATERIALS AND METHODS

Isolation and selection of bacteria: The chromium resistant bacteria were isolated from the sites contaminated with tannery effluent, through enrichment culture technique. The contaminated soil samples were collected from the tannery waste disposal site of a functional leather processing factory at Kanpur, leather hub city of India located in the state of Uttar Pradesh.

For the isolation of chromium-resistant bacteria, 1 g of contaminated soil samples was inoculated in 500 mL flasks containing 100 mL of Luria Bertani (LB) broth enriched with 50 mg L^{-1} of Cr(VI) (source: $K_2Cr_2O_7$) solution. The stock solution of Cr(VI) was filter-sterilized with 0.22 μm membrane filter (Millipore Corporation, Bedford, Massachusetts). The flasks were incubated at 37°C, at

200 rev min^{-1} (revolutions per minute). After 24 h of incubation, the chromium-resistant bacteria were isolated from the broth by a serial dilution and plating technique on LA (Luria agar) plates amended with 50 mg L^{-1} of Cr(VI). Further, bacterial colonies were purified on LA plates and nine pure bacterial colonies were preserved in glycerol stock at -20°C for long-term use.

The growth and Cr(VI) reduction ability of all nine pure isolates was checked in filter-sterilized (0.22 μm membrane filter) absolute tannery effluent (Table 1) containing 1.24 mg L^{-1} of Cr(VI) without any nutritional amendment. The experiment was set up in 500 mL flask containing 100 mL of filter sterilized absolute tannery effluent. The primary inoculum (starter culture) was prepared by inoculating a loop of bacteria in 50 mL of LB in 250 mL flasks at 37°C at 200 rev min^{-1} for 24 h. Each flask was inoculated with 0.1% (v/v) of primary inoculums of each bacterium separately. Cultures were incubated at 37°C at 200 rev min^{-1} for 24 h and optical density was observed at 600 nm for growth study.

For Cr(VI) reduction test, aliquots were withdrawn and centrifuged at 7000 rev min^{-1} for 5 min at room temperature and the supernatant were analysed for the residual Cr(VI). Hexavalent chromium was determined colorimetrically (l540) with a spectrophotometer (Spectronic 1001) with the *s*-diphenylcarbazide method (Bartlett and James, 1996), with a detection limit of 0.5 μg L^{-1}.

In this study, out of nine isolates, three isolates (B2, B4 and B9) were selected based on their ability to grow and reduce Cr(VI) in absolute tannery effluent without any nutritional amendment for further research study. The chromate tolerance range of selected isolates were checked by streaking pure colonies of each isolate on LA plates amended with 100-2000 mg L^{-1} of Cr(VI) and incubating the plates at 37°C for 10 days. Optimum pH (range: 5-11) and temperature (range: 30-45°C) for the growth of all the three isolates were studied in 100 mL of LB in 500 mL of flasks. pH of the media was adjusted by adding aliquots of either 1 M HCl or 1 M NaOH. Each flask was inoculated with 0.1% (v/v) of primary inoculum. Cultures were incubated at 37°C, at 200 rev min^{-1} for 24 h and optical density was observed at 600 nm.

Chromium reduction analysis: The Cr(VI) reduction ability of bacterial isolates at higher concentrations were examined in LB media (pH 8) amended with three different concentrations of Cr(VI) (50, 100 and 200 mg L^{-1}). The stock Cr(VI) solution of 1000 mg L^{-1} was prepared by dissolving $K_2Cr_2O_7$ in distilled water and the solution was filter-sterilized with 0.22 μm membrane filter. Different concentrations of Cr(VI), containing LB media(50, 100 and 200 mg L^{-1}), were prepared by mixing appropriate amounts of Cr(VI) stock solution and LB media (Camargo *et al.*, 2003).

For reduction tests, 100 mL of LB (containing 50, 100 and 200 mg L^{-1} of the Cr(VI) concentrations separately) in 500 mL flasks were inoculated with 2% (v/v) of starter culture and incubated at 37°C, at 200 rev min^{-1}. After 120 h of incubation, aliquots were withdrawn and centrifuged at 7000 rev min^{-1} for 5 min at room temperature and the supernatant were analysed for the residual Cr(VI) with the s-diphenylcarbazide method (Bartlett and James, 1996).

Effect of different media on growth and chromium reduction by isolates: The effect of three different media (pH 8.0) (LB, tannery effluent and AMM (acetate minimal media)) amended

Table 1: Chemical characteristics of tannery effluent

EC (mS cm^{-1})	pH	Cr(III) (mg L^{-1})	Cr(VI) (mg L^{-1})	Total P (mg L^{-1})	Total Kjeldahl N (mg L^{-1})	COD (mg L^{-1})
18.03	8	7.91±0.64	1.24±0.32	772.50±55.5	818±92.6	520

Values are as Mean±standard error

with 50 mg L^{-1} of Cr(VI) on bacterial growth and chromium reduction by the isolates was examined. The AMM comprises (per litre) 1.0 g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 0.001 g $FeSO_4.7H_2O$, 0.001 g $CaCl_2.2H_2O$, 5.0 g $CH_4COONa.3H_2O$, 0.5 g yeast extract and 0.5 g K_2HPO_4. Tannery effluent used was absolute without any nutritional amendments and was filter-sterilized with 0.22 μm membrane filter. The inoculum concentrations and growth conditions were maintained as described in the chromium reduction analysis. The biomass free control (abiotic control), in case of all the three media was used to detect any possible abiotic Cr(VI) reduction brought about by media components. The bacterial growth study (in terms of optical density at 600 nm) and Cr(VI) reduction tests were carried out after 120 h of incubation.

Time course study of bacterial growth and chromium reduction in AMM and tannery effluent: The time course study of growth and chromium reduction by bacterial isolates in tannery effluent and AMM (pH 8.0) amended with 50 mg L^{-1} of Cr(VI) was analysed. The inoculum concentrations and growth conditions were maintained as described in the chromium reduction analysis. Bacterial growth and Cr(VI) reduction tests were carried out after every 12 h of incubation, for a total of 120 h.

16S rRNA identification of bacteria: The three bacterial isolates were identified by partial 16S rRNA sequencing as follows. The bacterial isolates were grown on Luria agar for 24 h at 37°C. The bacterial colonies were then suspended in nuclease-free water. DNA was extracted from the suspension. Universal bacterial primers-GM3F (5'-AGAGTTTGATCMTGGCTCAG) and GM4R (5'-GGTTACCTTGTTACGACTT) were used for PCR (polymerase chain reaction) amplification of the 16S rRNA gene. A PCR master mix (25 μL) was prepared by adding 2.5 μL of reaction buffer, 5 μL of dNTPs, 0.5 μL of Taq© polymerase, 1 μL of $MgCl_2$, 2 μL each of forward and reversed primers and 2 μL of isolated DNA template.

The genomic DNA template was amplified with a 30-cycle PCR (initial denaturation at 95°C for 5 min, subsequent denaturation at 95°C for 1 min, annealing temperature at 50°C for 2 min, extension temperature at 72°C for 2.3 min and final extension at 72°C for 10 min). The PCR product was analysed on 2% agarose gel and purified with a Q1Aquick spin column (Qiagen) gel extraction kit, according to the manufacturer's instructions. The purified PCR product were sequenced by Lab India Instruments, Pvt Ltd (Gurgaon, India) on an automated multi-capillary DNA sequencer, ABI Prism 3130xl Genetic Analyser (Applied Biosystems, Foster city, CA, USA) using the Big Dye Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems). The partial 16SrRNA gene sequences were compared with known sequences in the NCBI database by using BLASTN to identify the most similar sequence alignment.

Statistical analysis: Triplicates were set up for each parameter that was tested. Completely randomized design was used and random sampling was carried out. The means of the three replicate values for all data in the experiments obtained were tested in a one-way analysis of variance, using Costat software (CoHort, Berkeley).

RESULTS

Out of nine isolates, only three isolates (B2, B4 and B9) were able to grow and reduce 1.24 mg L^{-1} of Cr(VI) below the detection limit within 24 h of incubation in filter-sterilized absolute

tannery effluent without any nutritional amendment. All three selected isolates were gram positive rods and could grow at a temperature range of 30-45°C, optimum temperature for growth being 37°C and pH range being 5-11, optimum pH for growth being 8 for all the three isolates. Isolate B2 could tolerate up to 1200 mg L^{-1} of hexavalent Cr and isolates B4 and B9 could tolerate up to 1500 mg L^{-1} of hexavalent Cr. All three isolates belong to the genus *Bacillus*. Isolate B2 related to *Bacillus subtilis* (NCBI Sequence Viewer, accession number EU334108.1) with 98% similarity, isolate B4 related to *Bacillus* sp. (NCBI Sequence Viewer, accession number FM208185.1) with 100% similarity and Isolate B9 related to another *Bacillus* sp. (NCBI Sequence Viewer, accession number FJ348004.1) with 98% similarity.

Chromium reduction by isolates: All three isolates were able to grow and reduce Cr(VI) in LB media amended with three different concentrations (50, 100 and 200 mg L^{-1}) of Cr(VI). At 50 mg L^{-1}, isolates B2 and B4 were statistically similar in percent reduction, with 74.1% (37.06 mg L^{-1}) and 73.14% (36.57 mg L^{-1}) reduction, respectively, after 120 h of incubation. Isolate B9, which was significantly different ($p<0.01$) in percent reduction from the other two isolates, showed 61.5% (30.75 mg L^{-1}) reduction after 120 h of incubation. At 100 mg L^{-1}, all the three isolates were significantly different ($p<0.01$) from each other and the maximum percent reduction was observed in isolate B4 which showed 73.41% (73.41 mg L^{-1}) reduction after 120 h of incubation. The other two isolates, B2 and B9 showed 42.15% (42.15 mg L^{-1}) and 60% (60 mg L^{-1}) reduction, respectively. At 200 mg L^{-1} isolate B4 was significantly different ($p<0.01$) in percent reduction from the other two isolates and showed 48.88% (97.76 mg L^{-1}) reduction. The other two isolates, B2 and B9 showed 40.75% (81.5 mg L^{-1}) and 39.39% (78.7 mg L^{-1}) reduction, respectively after 120 h of incubation. Out of the three isolates, isolate B4 was, in general, able to reduce higher concentration of Cr(VI) at all the three concentrations and found to be the most suitable isolate for chromate reduction at higher concentrations.

Effect of media on growth, chromium reduction and time course study: A comparative growth and Cr(VI) reduction by all the three isolates in three different media is presented in Table 2. Considering LB media as a positive control, all the three isolates showed higher growth and chromate reduction in tannery effluent than in AMM media, except isolate B2 which showed higher reduction in AMM media. Among the three isolates, isolate B4 showed significant ($p<0.01$) influence of the media on its reduction ability whereas isolate B9 kept the activity non-significantly different. In biomass free controls, there was no measurable changes in Cr(VI) concentrations detected after 120 h of incubation in case of all the three media.

The time course study of bacterial growth and chromium reduction in AMM and tannery effluent is presented in Fig. 1a-c. The growth study carried out in AMM showed that isolate B2 was

Table 2: Effect of different media on growth and Cr(VI) reduction by isolates

	Bacterial growth (optical density at 600 nm)			Percent Cr(VI) reduction		
Bacterial strains	LB	AMM	TE	LB	AMM	TE
B2	0.778±0.053^{a}	0.088±0.005^{c}	0.39±0.007^{b}	74.10±0.66^{a}	59.79±2.05^{b}	49.55±2.12^{b}
B4	1.336±0.063^{a}	0.101±0.013^{c}	0.76±0.016^{b}	73.14±1.68^{a}	47.80±1.65^{c}	61.54±2.17^{b}
B9	1.553±0.051^{a}	0.221±0.02^{c}	0.93±0.038^{b}	61.50±1.35^{a}	56.92±1.63^{a}	61.41±2.06^{a}

Values are as Mean±standard error. Means within a row followed by different superscript letters are significantly different according to Duncan's multiple range test ($p\leq0.01$)

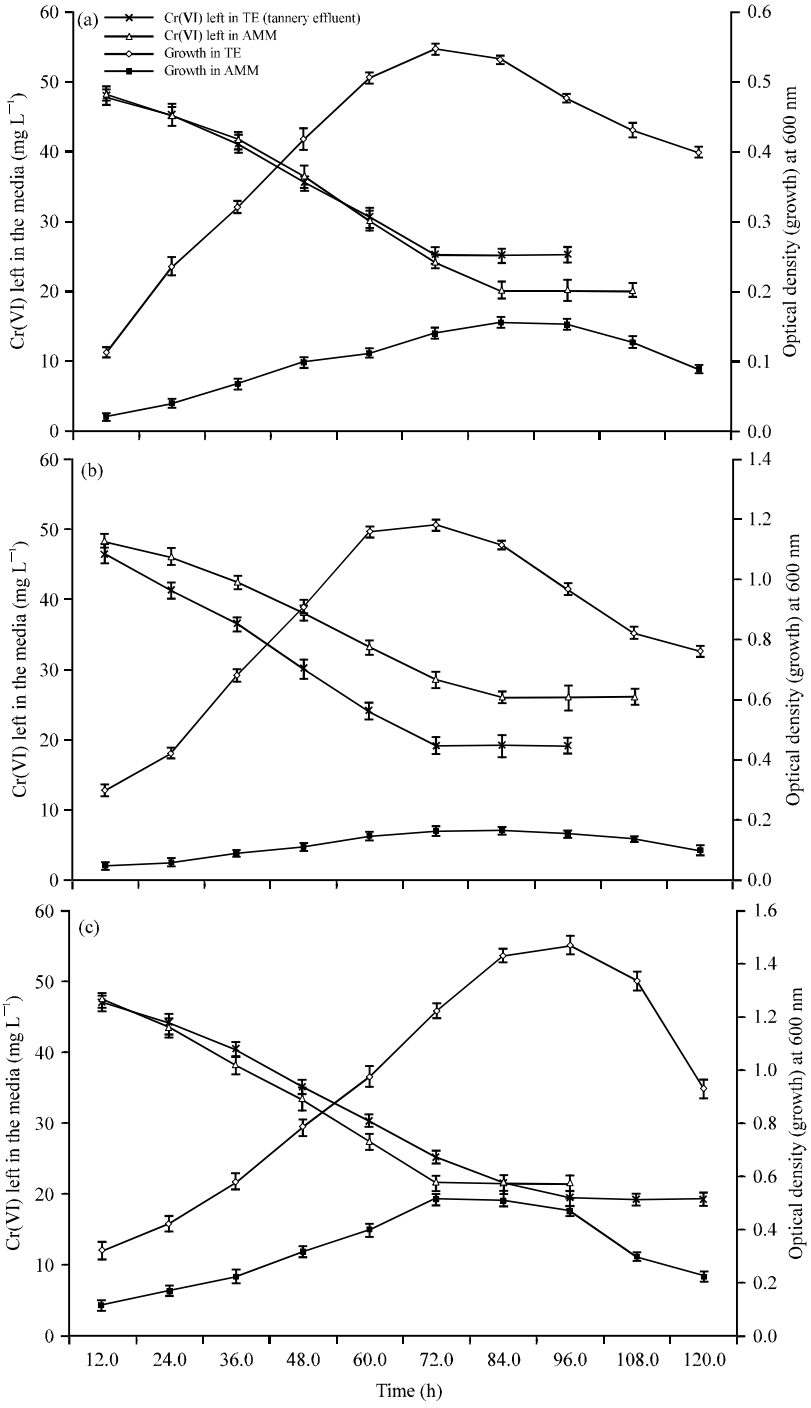

Fig. 1(a-c): Time course study of growth and Cr(VI) reduction by bacterial isolates in AMM (acetate minimal media) and tannery effluent amended with 50 mg L^{-1} of Cr(VI): (a) Isolate B2, (b) Isolate B4 and (c) Isolate B9. Error bars represent standard deviation of the triplicate experiment

able to grow up to 84 h of incubation. Isolates B4 and B9 were able to grow up to 72 h of incubation and after that, the cultures shifted to stationary and decline phases of growth. The maximum growth in AMM was shown by isolate B9 and least growth was shown by isolate B2.

The study carried out in tannery effluent showed that isolates B2 and B4 were able to grow up to 72 h of incubation and after that, the cultures shifted to stationary and decline phases of growth. Isolate B9 was able to grow up to 96 h of incubation. The maximum growth in tannery effluent was shown by isolate B9, which, interestingly, showed less growth than isolate B4 between 36 to 60 h of incubation. However, its growth increased between 72 and 96 h of incubation. The minimum growth in the tannery effluent was shown by isolate B2.

All the three isolates were able to reduce chromium in their active growth phase, that is, the reduction was directly proportional to growth. There was no further reduction in the stationary and decline phases of their growth. In case of the AMM, isolate B2 was able to reduce maximum amount (29.89 mg L^{-1}) of Cr(VI), with an average rate of 4.27 mg L^{-1} 12 h^{-1} (Fig. 1a). Isolate B4 was able to reduce minimum amount of Cr(VI) (23.903 mg L^{-1}), with an average rate of 3.42 mg L^{-1} 12 h^{-1} (Fig. 1b) and isolate B9 was able to reduce 28.52 mg L^{-1} of Cr(VI), with an average rate of 4.75 mg L^{-1} 12 h^{-1} (Fig. 1c). Out of three isolates, B9 showed maximum efficiency to reduce Cr(VI) within 72 h of incubation. Where as in case of the tannery effluent, isolates B4 (Fig. 1b) and B9 (Fig. 1c) were able to reduce approximately similar amounts of Cr(VI) (30.77 and 30.70 mg L^{-1}, respectively), with an average rate of 5.13 mg L^{-1} 12 h^{-1} and 3.83 mg L^{-1} 12 h^{-1}, respectively. Isolate B2 (Fig. 1a) was able to reduce the minimum amount (24.7 mg L^{-1}) of Cr(VI) with an average rate of 3.09 mg L^{-1} 12 h^{-1}. In tannery effluent, among the three isolates, isolate B4 showed maximum efficiency to reduce Cr(VI) within 72 h of incubation.

DISCUSSION

In this study, all three bacterial isolates were alkalophilic (pH 8) and could tolerate up to 1200 mg L^{-1} (B2: *Bacillus subtilis*) to 1500 mg L^{-1} (B4: *Bacillus* sp. and B9: *Bacillus* sp.) of hexavalent Cr. Detailed literature substantiated past work on Cr(VI) resistant microorganisms that are tolerant upto 10 to mg L^{-1} of Cr(VI) concentrations (Thacker *et al*., 2007; McLean and Beveridge, 2001; Basu *et al*., 1997). Few reports confirmed even higher tolerance range as high as upto 80 mg mL^{-1} by a *Bacillus* species (Shakoori *et al*., 2000). It is pertinent to mention that higher tolerance ability allows the bacteria to survive at higher concentrations of chromate but there is mostly no relationship towards its ability to reduce chromate therefore tolerance and reduction are two different mechanisms as reported by Pattanapipitpaisal *et al*. (2001) and Cervantes and Silver (1992).

The characteristic feature of micro-organism that not only survives in Cr(VI) concentration but also perform function towards its reduction is important and qualifying attribute for bioreduction. All micro-organisms need medium or source of food that provides nutrition which may be artificial/synthetic or naturally occurring for growth. The exhaustive literature search was conducted to distinctly indicate that tolerance and reduction ability of micro-organisms in artificial/synthetic media best achieved in diluted industrial effluents with modified nutrient and pH conditions (Ganguli and Tripathi, 1999; Acevedo-Aguilar *et al*., 2006). It was therefore necessary to find functional property of the micro-organisms that utilizes natural media such as soil or industrial effluent as a source of food. Such a function would be more vital and important if the natural medium is absolute, with its property not modified or amended or altered with added nutrition and pH conditions, respectively. The unique ability of bacteria to grow and reduce chromate in natural substrate such as tannery effluent without any amendment and alteration of physico-chemical property has been achieved under this research study. Selectively found bacterial isolate that is able to reduce chromate in the most conventional form of effluent proved for the first time to the best of our knowledge that no dilution or chemical alteration is needed. The study reports for the first time; the ability of three bacterial isolates, B2: *Bacillus subtilis*, B4:

Bacillus sp. and B9: *Bacillus* sp., to grow and reduce 1.24 mg L^{-1} of Cr(VI) in absolute tannery effluent without any nutritional amendment, below the detection limit with in 24 h. In a comparative growth and reduction study, all three isolates prefer tannery effluent over AMM for growth and reduction except isolate B2 which showed higher reduction in AMM. The isolates -B2, B4 and B9 showed 49.55% (24.7 mg L^{-1}), 61.54% (30.77 mg L^{-1}) and 61.41% (30.70 mg L^{-1}) reduction, respectively, in absolute tannery effluent without any nutritional amendment, within 72 h of incubation in the given 50 mg L^{-1} of initial Cr(VI) concentration. Out of three isolates studied, isolate B4 showed maximum efficiency (5.13 mg L^{-1} 12 h^{-1}) to reduce Cr(VI) in absolute tannery effluent. In growth media [containing 200 mg L^{-1} of Cr(VI), isolate B4 showed 97.76 mg L^{-1} of Cr(VI) reduction with in 120 h of incubation.

In recent past, various reports of indigenous fungi (Srivastava and Thakur, 2006; Acevedo-Aguilar *et al.*, 2006; Fukuda *et al.*, 2008; Morales-Barrera and Cristiani-Urbina, 2008) and bacteria, including *Bacillus* species (Sultan and Hasnain, 2000; Faisal and Hasnain, 2006; Philip *et al.*, 1998; Camargo *et al.*, 2003; Pal and Paul, 2004; Cheung and Ji-Dong, 2005; Thacker *et al.*, 2007; Quintelas *et al.*, 2008, 2009) isolated from tannery effluent, sludge and Cr-contaminated soil for Cr(VI) reduction/removal has been reported but all the reports of Cr(VI) reduction/removal are based on synthetic media or diluted industrial effluent, with modified nutrient and pH conditions. Acevedo-Aguilar *et al.* (2006) has reported 96% reduction of 50 mg L^{-1} of Cr(VI) by a *Penicillium* sp. after 72 h in an electroplating liquid waste diluted with nutrient media. Ganguli and Tripathi (1999) has reported the ability of an indigenous *Pseudomonas aeruginosa* strain to reduce 57.5% of Cr(VI) out of 40 mg L^{-1} in tannery effluent supplemented with the source of carbon, nitrogen and phosphorus after 36 h of incubation. In another study, Jeyasingh and Philip (2005) has reported 97% Cr(VI) reduction after 20 days by an indigenous bacteria in contaminated soil [containing 5.6 mg Cr(VI)/g of soil] amended with mineral medium and molasses as a carbon source. But in real terms, the ability of microbes to perform in synthetic media or in diluted industrial effluent with modified nutrient and pH conditions cannot be replicated in case of real industrial effluent conditions because of their major difference in the physico-chemical properties (Pattanapipitpaisal *et al.*, 2001; Srivastava and Thakur, 2006; Prigione *et al.*, 2009; Durai and Rajasimman, 2011) and on the other hand the addition of nutrients and modification of pH will inflate the cost economics and due to that it cannot be practically transformed into sustainable technology (Park *et al.*, 2005). If the developed technology is not cost effective, it cannot be adopted by small scale and unorganized sector industries which are the major sources of Cr(VI) contamination in the environment, especially, in poor and developing countries. In present study, we have studied the reduction ability of microbes in filter sterilized (0.22 μm membrane filter) absolute tannery effluent without changing its physico-chemical properties which is truly conventional effluent condition. Needless to say, the progress achieved through these novel bacterial isolates could provide a new basis to treat tannery effluent in a more efficient and sustainable way.

In general, chromate reduction by bacteria occurs under aerobic or anaerobic conditions and it has been associated with soluble or membrane-bound enzyme activities (Bopp and Ehrlich, 1988; Wang and Shen, 1990; Ishibashi *et al.*, 1990; Suzuki *et al.*, 1992). Several bacteria including *Bacillus* species, such as *B. coagulans*, *B. subtilis* and *B. megaterium*, isolated from Cr-polluted soil have been reported to act with soluble or membrane-bound chromate reductase enzyme (Campos *et al.*, 1995; Philip *et al.*, 1998; Garbisu *et al.*, 1998; McLean and Beveridge, 2001; Pal *et al.*, 2005; Cheung *et al.*, 2006). In the present study, reduction of chromate was not observed in biomass free controls re-confirming direct relationship between bacterial growth and Cr(VI) reduction in case of all the three studied isolates. Similar observation was reported by

Camargo *et al.* (2003) in case of *Bacillus* sp. and *Arthrobacter* bacteria. From the observation, it seems feasible that reduction of chromate by bacterial isolates results from cellular metabolism processes may be based on the reducing power of the organic carbon present in the effluent. Tannery effluent contains high levels of organic carbon in the form of proteins, carbohydrates, fats and oils (Sahu *et al.*, 2009). The role of fatty acids as a sole source of carbon for the growth of *Acinetobacter* and *Pseudomonas* spp. in metal working fluids (used in large scale industrial operations) has been reported by Foxall-Vanaken *et al.* (1986). The role of organic carbon in chromate reduction has been reported in *Pseudomonas* and *Enterobacter cloacae* bacteria (Ganguli and Tripathi, 1999; Ohtake *et al.*, 1990). In case of *Bacillus sphaericus* Pal and Paul (2004) has reported the reduction of 20 mg L^{-1} of chromate to 2.45 mg L^{-1} after 96 h of incubation in presence of glucose as a carbon source. From the study, it is clear that all the three studied bacteria have specific ability to utilize the raw form of nutrients that are already present in tannery effluent for its growth and chromate reduction.

In this study, all the three isolates were able to overcome the limitations, such as cell death, by high concentration of Cr(VI) and continuous supply of expensive nutrients or chemicals (White and Gadd, 1995; Al-Saraj *et al.*, 1999; Park *et al.*, 2005), which most of the studied micro-organisms in the past could not withstand (Ganguli and Tripathi, 2002; Srivastava and Thakur, 2006; Quintelas *et al.*, 2008, 2009). The study demonstrated that out of three isolates, isolate B4 (*Bacillus* sp.) has strong potential for the removal of chromium from the tannery effluent. With the ability to grow in the tannery effluent and reduce Cr(VI), this bacterium (*Bacillus* sp., B4) could be ideal for developing a sustainable green technology. The ease of growing these bacteria in tannery effluent, without any amendment, is paving the way to develop a technology, in which the bacteria will grow directly in effluents and perform the desired activity (Cr(VI) reduction), possibly in a cost-effective manner. The developed technology due to its cost-effectiveness can be easily adopted by small-scale and unorganized sector industries to detoxify the hexavalent chromium before discharging the effluent in the natural environment.

Although, these results suggest the possibility of treating industrial effluents using this bacterium, further experiments need to be carried out with the objective of optimizing the conditions, which would allow a more efficient removal of Cr from industrial wastewaters.

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