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Chromium-tolerant bacteria in diversified soil microbial community in the bank of tannery waste water discharging canal of East Calcutta, West Bengal

Piku Sen[1], Anandita Pal[2], Budhhadeb Chattopadhyay[1], D. Pal[3]

[1]*Government College of Engineering and Leather Technology, Block LB, Sector III, Salt Lake, Kolkata – 98, India*

[2]*Integrated M.Sc. Biotechnology,St. Xaviers' College, Calcutta, India*

[3]*Department of Zoology, Uluberia College, Howrah, India*

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Abstract

Studies have revealed that East Calcutta Wetlands harbor a variety of microbial population possessing diverse genetic characters with versatile enzymatic and metabolic activities. They carry out bioremediation of different toxins, pollutants, heavy metals etc. Due to the discharge of improperly treated effluents from tanneries and other industries in and around Calcutta City, carcinogenic chromium (Cr^{6+}) contamination of both surface water and ground water has been reported in the East Calcutta Wetland area. A few examples in microbial diversity in this area include Rhodococcus sp., Bacillus sp., Pseudomonus sp., Azotobacter sp., Aeromonas sp. etc. The primary objective of this study was to isolate and identify a potent chromate-reducing bacterial strain. Cr^{6+} analysis was done and the bacterial population was enumerated by analyzing soil samples from different locations. The majority of the chromate-resistant bacteria isolates from the tannery effluents enriched soil showed a minimum inhibitory concentration (MIC) of Cr6+ ranging from 50 to 750 mg l^{-1}. About 39.47% of the total 38 isolates of bacterial strains were able to grow at 200 mg l^{-1} Cr^{6+}. The potent Cr^{6+}-resistant isolates showed a very high tolerance level to 750 mg l^{-1} and were able to show 100% Cr^{6+} reduction up to 200 mg l^{-1} within 48 h. The present study conclusively demonstrates the ability of native microbial population present in tannery effluent to reduce Cr^{6+} compounds. Furthermore, all the isolates have shown great potential for bioremediation of Cr^{6+}-containing wastes. It is also reported that plant roots release some inorganic and organic compounds which aid the microbial community in the bioremediation of heavy metal pollutants in the soil. This approach permits the selection of bacterial strains which could be used for specific environmental cleanup operations.

***Corresponding Author:** D. Pal ✉ debasishpal60@yahoo.com

Introduction

Hexavalent Chromium [Cr (VI)], in the form of chromate ($CrO4^{2-}$) or dichromate ($Cr_2O_7^{2-}$) species, is a serious anthropogenic pollutant found in the discharged effluent from various industries and one the several risk driving contaminants found at unacceptable levels in ground water as well as in soil and sediments.

Chromium, the seventh most abundant element on earth, occurs in oxidation states ranging from Cr(II) to Cr(VI). The most chemically stable and common forms of chromium in the environment are the trivalent Cr (III) and hexavalent Cr (VI) species which differ significantly in their physicochemical properties and biological responsiveness (i.e. toxicity). Cr (VI) is a highly soluble and toxic non essential metal for most organisms with chronic chromate exposure leading to mutagenesis and carcinogenesis, whereas Cr (III) is sparingly soluble, relatively innocuous and essential micronutrient for mankind (Katz, S A *et al.*,1993; Carventes, C,*et al.*, 2001 etc).

Bioreduction of Cr(VI) can occur directly as a result of microbial metabolism (enzymatic) or indirectly, mediated by a bacterial metabolite (such as H_2S) (Losi *et al.*, 1994a). A number of chromium-resistant microorganisms have been reported, including *Pseudomonas* spp. (Alvarez *et al.*, 1999; *Microbacterium* (Pattanapipitpaisal *et al.*, 2001), *Desulfovibrio*, *Enterobacter* spp. (Wang *et al.*, 1990; Clark, 1994), *Escherichia coli* (Shen and Wang, 1993), *Shewanella alga* , *Bacillus* spp. (Campos *et al.*, 1995), and several other bacterial isolates (Basu *et al.*, 1997; Losi and Frankenberger, 1994). However, most of them have been isolated from tannery sludge, industrial sewage, evaporation ponds, or discharge water, or were purchased from culture collections.

Environmental clean up strategies for Cr(VI) removal involve physicochemical or biological detoxification. Major limitations of physicochemical process are the high energy inputs, different chemical treatments and generation of unnecessary sludge, reactive chemical species as secondary waste (Katiyar and Katiyar, 1997) etc. As an alternative, biological detoxification of Cr(VI) is reported to be more eco-friendly, capable of offering high potential for removal of Cr(VI) (Carventes, C *et al*, 2001; Camargo, F.A.O. *et al*, 2004; Kamaludeen, S.P *et al,* 2003). Bioreduction and biosorption of Cr(VI) using bacterial, fungal, yeast or plant biomass are among the most economic and eco-friendly strategies currently employed for removal of Cr(VI) by biological means (Quintelas, C *et al*,2006; Sierra-Alvarez, R 2007; Oliveira, E A *et al*, 2005;Ramirez – Ramirez, R *et al*, 2004).

In the perspective of the available research information, it has been felt that, proper isolation and characterization of Cr(VI) resistant bacteria from soil and understanding of the mechanism of chromium reduction process by bacterial isolates are of immense importance which is supposed to be supplemented by in-depth study on different physicochemical conditions influencing chromium reduction by bacterial isolates. It is also necessary to identify the mechanism behind the resistance and isolate and characterize enzymes and plasmid for reduction of hexavalent chromium. These findings can be implemented to find out the exact cause responsible for chromium reduction which in turn can be used in efficient biological treatment of tannery effluent.

The objective of this study was to isolate and characterize the culturable microbial community and further to evaluate the Cr(VI) resistance and hexavalent chromium reducing ability by highly tolerant strains, which can be used for biological treatment of chromium contaminated sludge.

Materials and methods

Site description

The East Calcutta Wetlands (lat. 22°33'–22°40'N; long.88°25'–88°35'E) used to receive untreated wastewater from at least 6000 different industrial units, including tanneries and premises dealing with rubber, electroplating, batteries, etc. and municipal sewage from Calcutta city. This complex wastewater

flows down through a web of canals into the wetland. The sampling site was selected on bank of such canal carrying wastewater.

Collection of samples

Soil sample was collected from three different points of a canal at East Calcutta Wetlands Area, carrying effluent discharge of tannery agglomerate in Calcutta, West Bengal. Bank soil samples collected in sterilized glass container were brought to the laboratory for microbial and chemical analysis in refrigerated condition.

Isolation of Chrome resistant bacteria

Soil suspensions were made with autoclaved distilled water. For the isolation and enumeration of the bacteria samples were serially diluted (dilution factor being10^{-1}, 10^{-2} and 10^{-3} respectively) and plated first on LB-Agar (pH 7.0). Plates were incubated at 37°Cfor 24 hrs. Colonies obtained were picked and purified by many rounds of re striking on LB agar plates amended with 100 mg/L $K_2Cr_2O_7$. From this preliminary screening strains showing resistance to chromium were selected for further studies. Slants were prepared from these isolates and stored at 4°C.

MIC Assay for Chromium Resistance Strains

Minimum Inhibitory Concentration (MIC) for hexavalent chromium was determined in LB medium containing different concentration of Cr (VI) i.e. 0, 50, 100,150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 and 1000 mg/L as $K_2Cr_2O_7$. 5 ml of Lb medium was inoculated with 500 µl of a fresh overnight culture in LB medium. Tubes without metal were used as control. All tubes were incubated with shaking at 37°C and 150 rpm. The growth of bacteria was monitored by turbidity (UV-VIS Spectrophotometer, Perkin Elmer).The lowest concentration of metal that completely preventing growth was determined as MIC.

Determination of Cr(VI) reduction ability

The chromate reduction capability of isolate was determined under aerobic condition in LB medium amended with 200 mg/L Cr (VI) as $K_2Cr_2O_7$. Broth medium was inoculated with 500µl of overnight culture. Tubes were incubated with shaking at 37°C and 150 rpm. Chromate reduction was measured at different time intervals. Cells were removed by centrifugation at 10,000 rpm and residual Cr (VI) in the supernatant was estimated.Cr (VI) was determined spectrophotometrically (Perkin Elmer) by 1, 5-diphenyl carbazide method. The Cr (VI) content in supernatant was estimated by adding 0.2ml orthophosphoric acid and 0.2 ml of acetone solution of 1, 5- diphenyl carbazide. The absorbance was measured at 540nm. (APHA, 1995).

Results and discussion

pH of the sample varied from 7.0 to 7.9. The total chromium concentration in soil sample was 36211 mg/kg (following *Issac and Kerber, 1971*). The total number of microorganisms in the soil samples showing Cr resistance was 38. 8 isolates were grown in 72 hrs, 5 isolates took 96 hrs and rests were grown in 24 hrs. Few bacterial growths on solid medium were higher than those in liquid medium. This may be due to the condition of diffusion. Complexation and availability of metals in liquid medium were different from those observed in solid medium. This observation is in agreement with Mergeay *et al* ,1995. MIC was done for all 38 isolates. Table 1 lists the MIC of 38 Cr resistant isolates.

MICs of 15 isolates ranged above 200 mg L^{-1} as $K_2Cr_2O_7$. PEc 33 showed maximum MIC out of these 38 isolates i.e. 750 mg L^{-1} as $K_2Cr_2O_7$and it was selected for further studies to determine the Cr(VI) reduction ability. Chromate reduction of PEc33 was monitored in LB broth with a Cr (VI) concentration of 200 mg/L as $K_2Cr_2O_7$ which was found to be reduced up to 12.76% after 6 hrs, 52.6% after 12 hrs and 100 % after 24 hrs. The reduction in our result suggests that PEc33 has the ability to reduce Cr (VI) under aerobic conditions. This study demonstrated that there are

several bacteria which has strong potential for removal of chromium from tannery effluent and could be ideal for developing a sustainable green technology, possibly in a cost effective manner.

Although strains showed MICs more than 200 mg L^{-1} as $K_2Cr_2O_7$ need to be identified biochemically.Future study will include the mechanism of Cr resistance of PEc 33 and optimization of conditions, which would allow a more efficient removal of Cr from industrial waste water by using these stains.

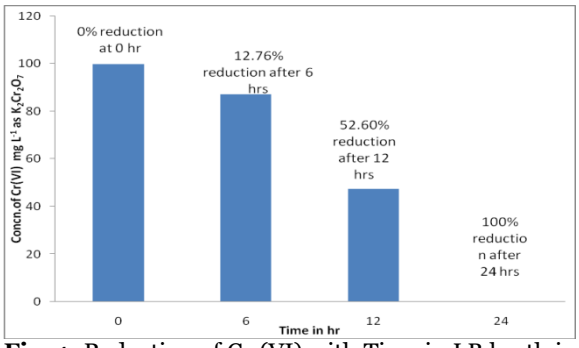

Fig. 1. Reduction of Cr (VI) with Time in LB broth in presence of PEc33.

Table 1. Minimum Inhibitory Concentration (MIC) of Cr to bacterial isolated from chromium contaminated soils from a cannal in East Calcutta Wetland carrying effluent from tanneries in Kolkata, West Bengal.

Strain Name	As $K_2Cr_2O_7$ (mg.L^{-1})	Strain Name	As $K_2Cr_2O_7$ (mg.L^{-1})
PEc 1	100	PEc47	50
PEc2	150	PEc49	100
PEc5	150	PEc51	200
PEc6	150	PEc53	150
PEc8	200	PEc57	200
PEc10	100	PEc58	100
PEc11	100	PEc60	100
PEc12	200	PEc61	150
PEc13	200	PEc62	150
PEc16	200	PEc64	150
PEc23	100	PEc69	150
PEc 24	200	PEc72	300
PEc29	200	PEc78	200
PEc32	300	PEc81	150
PEc33	750	PEc85	100
PEc34	250	PEc87	50
PEc35	150	PEc88	100
PEc36	150	PEc90	250
PEc38	200	PEc91	150

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