Hexavalent chromium reduction and 16S rDNA identification of bacteria isolated from a Cr (VI) contaminated site

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Citation

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Abstract

A Gram-positive, hexavalent chromium [chromate: Cr (VI)]-resistant & reducing bacterium, isolated from sukind chromite mines, jajpur, India, was identified as a Brevibacterium casei(Gene Bank Accession Number: EU781952) by gene sequence homology. The strain(designated as APD15) could tolerate chromium up to a maximum concentration of 500 ppm, at optimum temperature and pH 30C and 7 for maximum chromium reduction. Agar supplemented with 100g Cr (VI)/ml as $K_2Cr_2O_7$ and 0.5% (w/v) dextrose used as a carbon source. The results of the study indicated removal of more than 94% chromium (VI) by Brevibacterium caseidetermined by diphenylcarbazide colorimetric assay.

INTRODUCTION

Hexavlent Chromium is widely use in various industrial and viable processes, including mining, electroplating, leather tanning, petroleum refining, textiles inorganic chemicals and pulp production, and many other metal finishing industries (Wang and Xiao 1995) & is considered as a serious environmental pollutant. Chromium exists in the environment in several diverse forms such as trivalent [Cr (III)] and hexavalent [Cr (VI)], (Fendorf 1995) of which hexavalent chromium is a so-called carcinogen and a potential soil, surface water and ground water contaminant. Whereas it's reduced trivalent form ($Cr3^+$) is much less toxic, insoluble and a vital nutrient for humans. Cr (III) occurs naturally in the environment and is an essential nutrient required by the human body (Mishra & Das 2007). In India, Sukinda mines in Jajpur district of Orissa state witnesses a vast mining and mineral processing waste that are continuously discharged into open fields and are gradually becoming a source of Cr toxicity for human life, environment and animals and hence pose a serious threat to the inhabitants of this region. About 26 lakhs people residing on the banks of Brahmini River have fallen prey to water contamination due to Chromite mines discharged water which has been highlighted by the Blacksmith Institute. Hence there is an urgent need to reduce Cr (VI) contamination in this region. Recently, bioremediation of Cr (VI) has gained considerable consideration (Wang et al. 1989; Yamamoto et al. 1993; Middleton et al. 2003). Some microbial species can utilize Cr (VI) as a terminal electron acceptor in their respiratory process and transform Cr (VI) to less toxic Cr (III) compounds (Lovley and Phillips 1994; Shen et al. 1996). A number of these microorganisms, particularly bacteria, can reduce Cr and therefore detoxify it (Fuji et al. 1990). The present study describes a microbiological treatment for industrial effluent that may be suitable for processing Cr-contaminated waste. This study proposes a remediation route for detoxification of Cr (VI) using an indigenous microorganism.

MATERIALS AND METHODS

BACTERIAL STRAINS AND GROWTH CONDITIONS

Four strains used in this study were originally isolated by Mr. A.P. Das sukind chromite mines, jajpur, India. Bacterial strains, resistant to Cr (VI), were isolated from the soil using the serial dilution technique in PYE medium (Peptone, Yeast extract). Agar supplemented with 100g Cr (VI)/ml as $K_2Cr_2O_7$ and 0.5% (wt/vol) dextrose served as carbon source. The pH was maintained at 7±0.2 by using HCl or NaOH. The isolates are tested for their chromate tolerance at different concentrations (12.5, 25, 50, 75, 100 l/ml) of hexavalent chromium supplemented as $K_2Cr_2O_7$. Significant growth of the specific bacterial species in the presence of 100 mg Cr (VI)/l in PYE medium during two-day incubation

at 30°C, were considered as Cr (VI) resistant. A single strain was capable of growing at this condition & was selected for further experiments.

CR (VI) ANALYSIS

Chromate-reducing activity was estimated as the decrease in chromate concentration in supernatant with time using the Cr(VI)-specific colorimetric reagent 1,5-diphenylcarbazide (DPC), prepared in acetone/H2SO4 to minimize deterioration (Urone 1955) as follows: DPC (0.025 g) was dissolved in 9.67 ml acetone (AR) and 330 µl of 3 M H2SO4 was added. The reaction mixture was set up in an Eppendorf tube containing the following: 200 µl sample or standard sodium chromate solution, 400 µl 20 mM MOPS-NaOH buffer pH 7.0, 33 µl 3 M H2SO4, 40 µl 0.25% (w/v) DPC, and 327 µl distilled water. Spectrophotometric measurements were made immediately at 540 nm.

IDENTIFICATION OF SELECTED STRAINS

Gram staining of bacterial strain was carried out using established methods (Collins and Patricia 1984). For PCR amplification, a small amount of a bacterial colony was resuspended in 100 µl of sterile deionised water (SDW), mixed and lysed at 70°C (10 min). Crude lysate (0.2 µl) was added to 19.8 µl SDW and used as a PCR template. Universal bacterial 16S rDNA gene primers pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and pH′ (5′-AAGGAGGTGATCCAGCCGCA- 3′) were used to amplify the ~1.5 kb 16S rDNA gene fragment (Edwards et al. 1989). Sequence data was aligned and analyzed for finding the closest homology for the microbe.

PRELIMINARY SCREENING FOR CR (VI)-REDUCING ACTIVITY

Strain was grown aerobically in PYE at 30°C overnight. For anaerobic Cr(VI) reduction tests, 45 ml of PYE in 50 ml serum bottles was degassed with O2-free N2 (10–20 min). Each bottle was inoculated using a syringe with 10% (v/v) of the starter culture and incubated statically at 30°C. Potassium dichromate (100 µM) was added after 2 h. Samples (1 ml) were withdrawn periodically and the bacterial density was determined as OD600 prior to harvest by centrifugation at room temperature. The cell pellet was suspended in 1 ml of isotonic saline [0.85% (w/v) NaCl] for protein assay and the supernatant was assayed for residual Cr (VI).

EFFECT OF INITIAL CR (VI) CONCENTRATION AND INITIAL CELL DENSITY ON CR (VI) REDUCTION BY STRAIN APD15

Strain APD15 (deposited in the National Center for Biotechnology Information (NCBI) Genbank; accession no. EU781952) was precultured aerobically as described above. For aerobic Cr (VI) reduction tests, 45 ml of PYE in 250 ml flasks was inoculated with 10% (v/v) of the primary inoculum and incubated with shaking at 30°C with parallel anaerobic tests as described above but with various concentrations of sodium chromate added immediately after incubation. Growth, and residual chromate in the sample supernatants, was determined as above. To determine the effect of initial cell density, cells were precultured aerobically (30°C, 24 h) in PYE (40 ml). Cells were harvested by centrifugation at 4°C (15 min), and kept on ice until use (usually within 1 h). Anaerobic Cr(VI) reduction tests were performed as described above with initial cell densities of 2.7×106, 5.3×107, 1.4×108, 1.0×109, and 2.4×109 cells/ml. potassium dichromate was added to 100 µM and the cells were incubated at 30°C. Residual chromate in the sample supernatants was determined as described above.

RESULTS AND DISCUSSION

CR (VI)-REDUCING ACTIVITY OF ISOLATES

The Cr (VI)-reducing activity of 4 isolates was investigated as shown in Table 1. Strains APD1, APD8, APD15, and APD39 were selected for further study, with a maxi-mum removal of 94% of the Cr (VI) (APD15). These bacteria were Gram-positive, non spore-forming rods. On PYE agar, colonies were 1–3 mm in diameter, circular, low convex with an entire margin, opaque, and moist.

Figure 1

Table 1: Loss of hexavalent chromium [Cr (VI)]

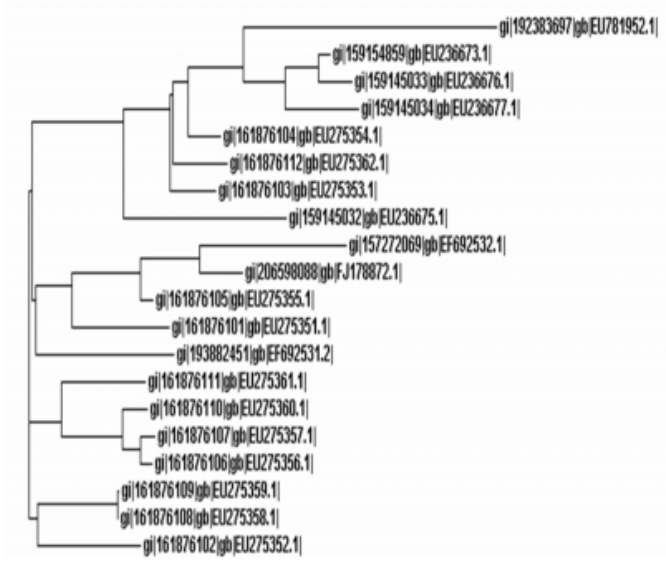
Isolate no.	Loss of Cr (VI) (%)
APD1	83.2
APD8	76.4
APD15	94.2
APD39	88.1

16S RNA IDENTIFICATION OF SELECTED STRAINS

Based on nucleotides homology and phylogenetic analysis the Microbe (Sample: APD15) was detected to be Brevibacterium casei (GenBank Accession Number: EU781952).The 16S rDNA nucleotide sequences from NCBI gen bank were compared with known sequences in the EMBL database using ClustalW2 to identify the most similar sequence.

Figure 2

Fig 1: phylogenetic tree of with similar sequence from NCBI genbank



CHROMATE REDUCTION ACTIVITY BY BREVIBACTERIUM CASEI (APD15)

The inoculum of the bacterial strains cultured overnight was used for this experiment. Culture flasks (150 ml) with a final volume of 100ml supplemented with (10-50mg/L) of Cr (VI) were inoculated with 2ml of inoculums for 24 hour. The growth kinetics of bacteria is characterized as initial lag phase, second exponential phase, stationary phase and death phase. In this experiment it is observed that lag phase is increasing with increased initial Cr (VI) concentration [Fig 2 & 3]. It is basically due to inhibitory effect of higher chromium concentration on the growth of the organism. Each organism has a specific resistance at a specified growth condition. As the initial age of the inoculum was fixed at 24 hours the acclimatization period at varying chromium concentration will not remain same. Hence the following behavior is observed. The chromium-resistant bacteria isolate exhibited reduced bioaccumulation when cells were in stationary phase. At higher concentrations the growth of the bacteria is inhibited due to fixed amount of inoculum for all the different concentration of Cr (VI) considered in the experiment.

Figure 3

Fig-2: Cr (VI) degradation kinetics varying chromium concentration

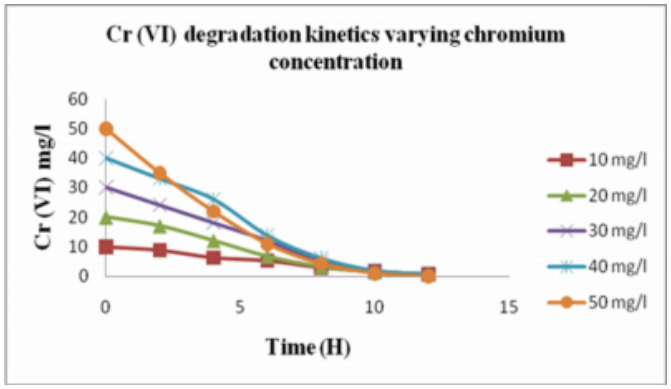
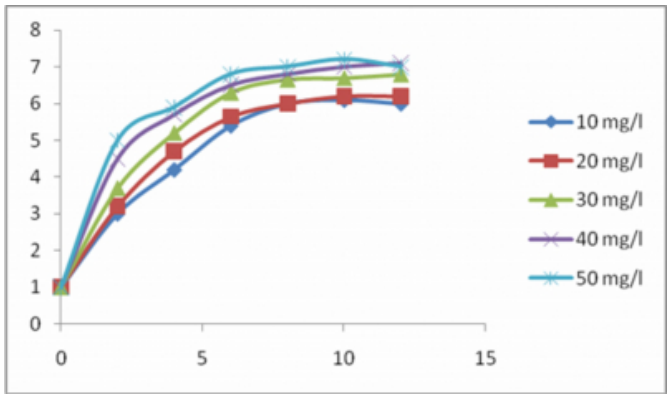


Figure 4

Fig-3: Biomass growth at varying chromium concentration



CONCLUSION

Bacterial chromate reduction has been reported under aerobic (Ishibashi et al. 1990; Cooke et al. 1995; Wang and Xiao 1995), anaerobic (Romanenko and Koren'kov 1977; Lebedeva and Lyalikova 1979), or both (Llovera et al. 1993; Shen and Wang 1993) conditions. In this study, a newly isolated Cr (VI)- reducing bacterium was identified as a Brevibacterium sp. This bacterium reduced Cr (VI) anaerobically at the expense of Peptone & Yeast extract as the source for growth. Wang and Xiao (1995) studied the effect of Cr(VI) concentration (100–500 M) on the aerobic reduction of Cr(VI) by Bacillus sp. and P. fluorescens LB300 at an initial cell concentration of 1010 cells/ml, reporting that complete Cr(VI) reduction by Bacillus sp. was not observed for concentrations higher than 100 M in 96 h. With P. fluorescens LB300, complete Cr (VI) reduction did not occur even at the lowest Cr (VI) concentration during the same period. However, many researchers have suggested that bacterial chromate reduction and resistance are independent processes (Ohtake et al. 1987; Bopp and Ehrlich 1988), hence selection for chromate resistance is not usually considered as an appropriate strategy to select for Cr (VI)-reducing strains. However, Brevibacterium sp. APD15 is unusual because it is resistant to Cr (VI) during growth while being able to reduce Cr (VI).These experiments concluded that the cells have a elevated ability to reduce Cr (VI) coupled with resistance of the Putative metal reductase to toxic chromate and the Cr (III) product. These findings are potentially useful because this bacterium could be harnessed to the detoxification of chromate-contaminated industrial & mining waste by growth in the waste solution aerobically followed by an anaerobic reductive step, with the potential for biomass regeneration in a second aerobic cycle. This potential, and the resistance mechanism, are the subject of ongoing studies.

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