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Cytosolic Reduction of Toxic Cr (VI) by Indigenous Microorganism

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Abstract: In the present study chromium resistant bacterial strain *Bacillus cereus* S-6 isolated from effluents of tannery was used for the reduction of toxic Cr (VI) into less toxic Cr (III). It could resist very high concentration of K_2CrO_4 i.e., up to 40 mg mL^{-1} on nutrient agar, 25 mg mL^{-1} in nutrient broth and up to 1.5 mg mL^{-1} of K_2CrO_4 in acetate-minimal medium. At an initial Cr (VI) concentration of 100 μg mL^{-1}, the cytosol and membrane preparation of the strain were able to reduced almost 67 and 43% of Cr (VI) within 24 h incubation period while the heat killed cytosol and membrane preparation reduced 24 and 18% within the same time period. At high initial K_2CrO_4 concentration (500 μg mL^{-1}), the reduction percentage decreased and cytosol reduce 36% and membrane preparation 18% of the total chromium supplied after 24 h. After heat shock these reduction values were 13 and 9%, respectively.

Key words: Bioremediation, Cr (VI) reduction, *Bacillus cereus*, heavy metals

INTRODUCTION

Increased industrialization posed harsh effects on the environment through the rough elimination of heavy metals containing wastes. Metals like nickel, cadmium, lead, chromium, copper, mercury, uranium etc. found in waste waters are harmful to the environment and their effects on biological system are too severe (Panchal *et al*., 2006). Chromium occurs in oxidation states Cr (0) to Cr (VI) but two forms Cr (III) and Cr (VI) are biologically important. Trivalent chromium, Cr (III) is an essential element required for normal carbohydrate and lipid metabolism (Mihaela *et al*., 2005). Several factors make Cr (VI) contamination as a matter of intense concern, particularly its toxic, mutagenic (James *et al*., 2005), carcinogenic (Max and Klean, 2006) and terartogenic effects above critical level (Asmatullah *et al*., 1998). Removal of such toxic metal by its reduction to the much less harmful and physically immobile state is now our concern. Several bacterial strains (*Pseudomonas ambigua*, *Desulfovibrio vulgaris*, *Enterobacter cloacae* HO-1, *Alcaligenes eutrophus*, *Dinococcus radiodurans* R1) has been described for their ability to reduce hexavalent chromium into insoluble low valence form Cr (III) both aerobically and aerobically (Branco *et al*., 2004; Dmitrenko *et al*., 2006; Elangovan *et al*., 2006). Hence the chromate reducing bacteria are most crucial for the immobility of soluble hexavalent chromium in the environment. The goal of present study was to develop a system to describe chromate reduction alone and in the presence of other toxic contaminants.

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MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Bacterial strain *Bacillus cereus*, used in this study was isolated and identified by Faisal and Hasnain (2004). The chromate resistance level in this strain was checked both in nutrient as well as in acetate-minimal media (Pattanapipitpaisal *et al*., 2002) supplemented with different concentrations of K_2CrO_4.

Cr (VI) Reduction Experiments

In this study two initial K_2CrO_4 concentrations (100 and 500 μg mL^{-1}) were used. Overnight culture of *Bacillus cereus* was taken and washed thrice with saline water and the resultant pellet was sonicated. The mixture was centrifuged at 10,000 rpm for 10 min. Supernatant (cytosol) and pellet (membrane preparation) were taken to check chromium reduction ability before and after heat shock at 121°C for 15 min. For reduction experiments, DeLeo and Ehrlich (1994) medium (grams per litre: tryptone 10, yeast extract 5, NaCl 5, citric acid 1, Na_2HPO_4 6.9) was used. After 24 h incubation period samples were taken aseptically and were analyzed for Cr (VI) reduction. Chromium (VI) reduction was monitored with the help of a spectrophotometer at 540 nm using diphenylcarbazide as complexing agent following method of Clesceri *et al*. (1998).

Statistical Analysis

Standard errors of the means and LSD were calculated following Steel and Torrie (1981).

RESULTS

Present study was conducted in 2005-2006 at Microbiology and Molecular Genetics Research Lab, Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan where a chromium resistant/reducing bacterial *Bacillus cereus* was examined for its capacity to reduced hexavalent chromium.

Isolation, Characterization and Identification of Strain

The present work deals with bacterial strain *Bacillus cereus*. *Bacillus cereus* was isolated from chromium contaminated soil sample from Sheikhupura, Pakistan (Faisal and Hasnain, 2004). It could resist very high concentration of K_2CrO_4 in nutrient broth (up to 25 mg mL^{-1}) as well as on nutrient agar (up to 50 mg mL^{-1}). It is a spore former motile strictly aerobic rod.

Cr (VI) Reduction

In this experiment the reduction of Cr (VI) by the cytosol (supernatant) and pellet (membrane preparation) after the sonication of the bacterial cell pellet before heat shock and after heat shock were observed after 24 h incubation. Two initial K_2CrO_4 concentrations were used i.e., 100 and 500 μg mL^{-1}. The reduction of hexavalent chromium by the *Bacillus cereus* in medium resulted in the production of offwhite residues that was sign of chromate reduction. The strain exhibits variation in reduction potential of hexavalent chromium. At an initial Cr (VI) concentration of 100 μg mL^{-1}, the supernatant and pellet of the strain was able to reduced almost 67 and 43% of Cr (VI) within 24 h incubation period. With an increase in K_2CrO_4 concentration (500 μg mL^{-1}) a decrease in Cr (VI) reduction was observed both in supernatant and pellet (36 and 18%, respectively) of the total chromium supplied after 24 h (Fig. 1). Figure 2 showed that after giving heat shock,

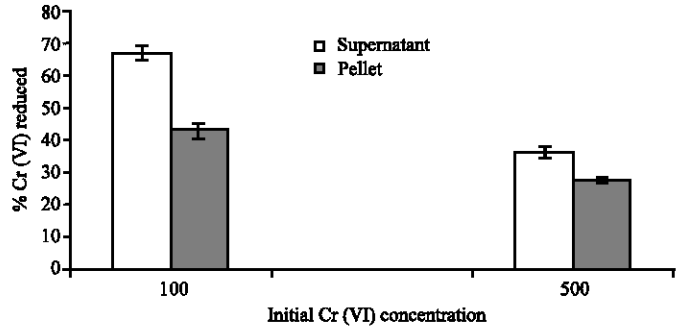

Fig. 1: Percentage Cr (VI) reduction in the supernatant (cytosol) and pellet (membrane preparation) of *Bacillus cereus* at an initial Cr (VI) concentrations of 100 and 500 μg mL^{-1}

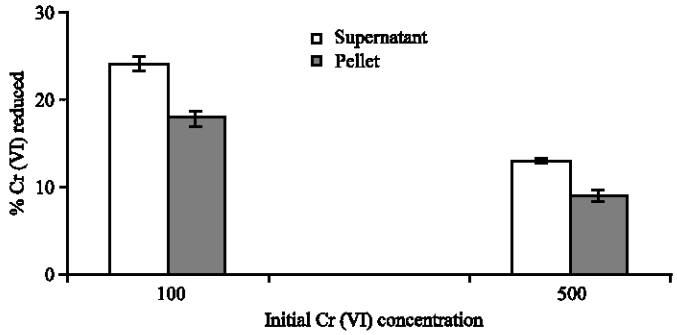

Fig. 2: Percentage Cr (VI) reduction in the supernatant (cytosol) and pellet (membrane preparation) of *Bacillus cereus* after giving heat shock at 121°C for 15 min. The initial Cr (VI) concentrations used were 100 and 500 μg mL^{-1}

the heat killed supernatant and pellet was able to reduced 24 and 18% at an initial K_2CrO_4 concentration 100 μg mL^{-1} after 24 h incubation. At high K_2CrO_4 concentration (500 μg mL^{-1}), supernatant reduced 13% and pellet 9% of the total chromium supplied after 24 h (Fig. 2).

DISCUSSION

Wide growing of industrial activities has lead to widespread chromium contamination within soils and water. Chromate is a widespread contaminant that has deleterious impacts on human health, the mobility and toxicity of which are diminished by reduction to Cr (III) (Elangovan *et al*., 2006). *Bacillus cereus* showed resistance to very high level of K_2CrO_4 both in nutrient broth (Up to 25 mg mL^{-1}) and on nutrient agar (50 mg mL^{-1}). Such a high level resistance was not reported previously in the literature. The chromate resistance level of this strain is also very high in acetate-minimal medium relative to the strains reported by other researchers. Chromium resistant bacteria isolated by Basu *et al*. (1997) from effluent of tanneries were resistant up to 250 μg mL^{-1} of Cr (VI). Megharaj *et al*. (2003) also isolated bacterial strains from chromium-contaminated soil which were able to grow at a concentration of up to 100 μg mL^{-1} of Cr (VI) in minimal medium. Most Cr (VI)-resistant microorganisms are tolerant up to 10 to 1500 mg L^{-1} of Cr (VI) (McLean and Beveridge, 2001). In the present study, besides Cr (VI), strains also have a broad ranges of heavy metals (Mn, Ni, Zn, Pb, Cu, Co and Hg) and antibiotics (streptomycin, ampicillin, kanamycin, tetracycline and chloramphenicol)

resistances (data not shown), which shows a positive sign for the successfulness of this strain in the treatment of industrial effluents. However, if this strain exhibit sensitive behavior (toward different metals and antibiotics), then first of all there must be a primary system to remove other metals contaminants before the treatment of target metal with these strains.

The Cr (VI) concentrations also affect the Cr (VI) reduction. In this case at two different K_2CrO_4 concentrations 100 and 500 μg mL^{-1} were used. Chromium (VI) reduction in supernatants and pellets before and after heat shock was observed. Cr (VI) reducing activity was found both in the supernatant and pellet of the bacterial cells. The cell free extract of *Bacillus* sp. reduced 50% Cr (VI) under anaerobic conditions, using NADH as an electron donor and produced a soluble a chromate reducing enzyme stimulated by copper Cu^{+2} (Camargo *et al*., 2003). In the present study highest Cr (VI) reduction potential was observed in supernatant (cytosol) at an initial Cr (VI) concentration 100 μg mL^{-1}. In anaerobic systems, membrane preparations reduce Cr (VI) and Cr (VI) has been shown to serve as a terminal electron acceptor (Lovley and Phillips, 1994). Assays with permeabilized (treated with toluene or triton x 100) cells and crude extracts verified that the Cr (VI) reduction was mostly associated with the soluble protein fraction of the cell (Megharaj *et al*., 2003). An enrichment consortium almost completely (98.5%) reduced 0.6 mM Cr (VI) in 168 h and rate of reduction was 0.5 g Cr (VI) g $protein^{-1}$ h^{-1} (Cheung *et al*., 2003). *Microbacterium* sp. at a concentration of 2.4×10^9 cells mL^{-1}, 100 μM sodium chromate was reduced within 30 h however; the maximum specific reduction rate was obtained at lower initial cell concentrations (Pattanapipitpaisal *et al*., 2001). After giving heat shock supernatant and pellet also retain their capability of Cr VI) reduction. In this case supernatant (cytosol) reduced maximum chromium as compared to pellet (membrane preparation). It was observed that Cr (VI) activity was lost when the Cr (VI)-reducing cell extracts were heated at 100°C (Ohtake and Hardoyo, 1992). When the cytosol and membrane was heat shocked at 121°C for 15 min, the Cr (VI) reduction property was drastically decreased. It means that the chromium reduction ability of this strain is related with some chromate reductase enzyme, which lost its activity after giving heat shock.

REFERENCES

Asmatulla, S.N. Qureshi and A.R. Shakoori, 1998. Hexavalent chromium induced congenital abnormalities in chick embryos. J. Applied Toxicol., 18: 167-171.

Basu, M., S. Bhattacharya and A.K. Paul, 1997. Isolation and characterization of chromium resistant bacteria from tannery effluent. Bull. Environ. Contam. Toxicol., 58: 535-542.

Branco, R., M.C. Alpoim and P.V. Morais, 2004. *Ochrobactrum tritici* strain 5bvl1- characterization of a Cr (VI)-resistant and Cr (VI)-reducing strain. Can. J. Microbiol., 50: 697-703.

Camargo, F.A., B.C. Okeke, F.M. Bento and W.T. Frankenberger, 2003. *In vitro* Reduction of hexavalent chromium by a cell free extract of *Bacillus* sp. ES 29 stimulated by Cu^{2+}. Applied Microbiol. Biotechnol., 62: 569-573.

Cheung, K.H. and J.D. Gu, 2003. Reduction of chromate CrO_4^{-2} by an enrichment consortium and an isolate of marine sulphate reducing bacteria. Chemosphere, 52: 1523-1529.

Clesceri, S.L., A.E. Greenberg and A.D. Eaton, 1998. Standard Methods for the Examination of Water and Waste Water, 20th Edn. American Public Health Association. Washington, DC.

Deleo, P.C. and H.L. Ehrlich, 1994. Reduction of hexavalent chromium by *Pseudomonas fluorescens* LB 300 in batch and continuous cultures. Applied Microbiol. Biotechnol., 40: 756-759.

Dmitrenko, G., V. Konovalova and T. Ereshko, 2006. The successive reduction of Cr (VI) and NO_3^- or Mn (IV) ions present in the cultivation medium of denitrifying bacteria. Microbiology, 75: 125-128.

Elangovan, R., S. Abhipsa, B. Rohit, P. Ligy and K. Chandraraj, 2006. Reduction of Cr (VI) by a *Bacillus* sp. Biotechnol. Lett., 28: 247-252.

Faisal, M. and S. Hasnain, 2004. Comparative study of chromium uptake and reduction in industrial effluent by *Ochrobactrum intermedium* and *Brevibactrium* sp. Biotechnol. Lett., 26: 1623-1628.

James, A., L. Stephen, R. Jenny, S.L. Claudia, Y. Shih-Houng, S. Xianglin and T. Michael, 2005. Effect of stainless steel manual metal arc welding fume on free radical production, DNA damage and apoptosis induction. Mol. Cellu. Biochem., 279: 17-23.

Lovely, D.R. and E.J.P. Phillips, 1994. Reduction of chromate by *Desulfovibrio vulgaris* and its C (3) cythochrome. Applied Environ. Microbiol., 60: 726.

Max, C. and K. Catherine, 2006. Toxicity and carcinogenicity of chromium compounds in humans. Crit. Rev. Toxicol., 36: 155-163.

Mclean, J. and T.J. Beveridge, 2001. Chromate reduction by a Pseudomonad isolated from a site contaminated with chromated copper arsenate. Applied Environ. Microbiol., 67: 1076-1084.

Megharaj, M., S. Avudainayagam and R. Neidu, 2003. Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste. Curr. Microbiol., 47: 51-54.

Mihaela, B., O. Rodica, V. Gina, E. Ana, P. Veronica and M. Dana, 2005. Thermal behavior of some new chromium complexes with potential biological importance. J. Therm. Analy. Calorimetry, 81: 273-276.

Ohtake, H. and J.K. Hardoyo, 1992. New biological method for detoxification and removal of hexavalent chromium. Water Sci. Technol., 25: 395.

Panchal, P.K., P.B. Pansuriya and M.N. Patel, 2006. Study on increase in toxicity of Schiff bases on microorganism on chelation with metal. Toxicol. Environ. Chem., 88: 57-64.

Pattanapipitaisal, P., A.N. Mabbett, J.A. Finaly and A.J. Beswick, 2002. Reduction of Cr (VI) and bioaccumulation of chromium by gram positive and gram negative microorganisms not previously exposed to Cr stress. Environ. Technol., 23: 731-745.

Steel, R.G.D. and J.H. Torrie, 1981. Principles and Procedure of Statistic. A Biometrical Approach. 2nd Edn., McGraw Hill Int., Book Company.