

RESEARCH ARTICLE

Isolation of chromium resistant *Bacillus* sp. MRKV and reduction of hexavalent chromium potassium dichromate

J. Chandhuru¹, S. Harshitha¹, K. Sujitha¹ and D.J. Mukesh Kumar²

¹Dept. of Biotechnology, St. Joseph's College of Engineering, Chennai, TN, India

²Centre for Advanced Studies in Botany, Maraimalai Campus, University of Madras, Chennai, TN, India
itsmemukesh@gmail.com; +91 9884553310

Abstract

The ability of *Bacillus* sp. MRKV isolated from tannery effluent which shows resistant towards hexavalent chromium potassium dichromate ($K_2Cr_2O_7$) was evaluated in this study. The bacterial strain was assayed for its reducing ability towards $K_2Cr_2O_7$. The study revealed that the isolated strain has the ability to reduce the hexavalent chromium and the optimal conditions required for the chromium reduction was also evaluated. The observations infer that the bacterial strains can be exploited for bioremediation of hexavalent chromium containing wastes, as they seem to have a great potential to reduce the toxic hexavalent form to a less toxic trivalent form.

Keywords: *Bacillus* sp., tannery effluent, hexavalent chromium, less toxic trivalent form.

Introduction

Heavy metals have vast industrial applications due to their technological importance. Chromium is the seventh most abundant element on earth and exists in several oxidation states. The most prevalent forms of chromium in the natural environment are hexavalent and trivalent (Chung *et al.*, 2006). Chromium is used in various industries: wood preservation, leather tanning, textile and ceramics. Wastewaters from these industries possess several toxic effects to life forms and the environment. Accumulation of these toxic metals in human has several adverse actions such as growth and developmental abnormalities, carcinogenesis, mental retardation and wide range of other illnesses (Thiele, 1995). In addition to these, the chromium and salt ions present in these waste waters have toxic effects towards the microbial consortia of wastewater treatment systems (Stasinakis *et al.*, 2003).

The available traditional methods for the removal of this toxic metal from industrial effluents include chemical precipitation, reverse osmosis, ion exchange, filtration, membrane technologies, evaporation recovery, etc. (Ahluwalia and Goyal, 2007). These processes were found to be less effective and extremely expensive (Nourbakhsh *et al.*, 1994). Therefore, it is mandatory to develop an innovative, cost-effective and eco-friendly method for removal of toxic chromium from the wastewater. The microbes present in such wastewaters will develop a resistance towards the toxic chromium metal. The mechanisms behind their resistance include adsorption, uptake, methylation, oxidation and reduction of toxic, highly soluble Cr^{6+} to less soluble and less toxic Cr^{3+} (Elangovan *et al.*, 2006; Goulhen *et al.*, 2006; Viamajala *et al.*, 2007).

The present investigation would aim at the isolation of chromium resistant bacterial strain from chromium contaminated industrial effluent, its characterization, reduction of hexavalent chromium and optimization of various parameters for chromium reduction.

Materials and methods

Isolation of chromium resistant bacteria: The effluent samples were collected from the dumping site of the Alfa Impex Tannery Industry, Chennai, in sterile containers and brought to the laboratory for further analysis. The bacterial isolates were allowed to spread on fresh, sterile Luria Bertani (LB) agar medium incorporated with 5 mg/L concentration of potassium dichromate (Rehman *et al.*, 2008). The medium was incubated for 3–5 d and the colonies differing in their morphological characteristics showing maximum growth were analyzed for further investigations.

Biochemical characterization of isolated bacteria: Purification of the bacterial culture was confirmed by Gram staining. The pure colonies were again cultured on Nutrient agar. After obtaining pure culture, following biochemical tests were performed for the preliminary characterization purposes: Indole test, Methyl red test, Vogler Proskauer test and Citrate utilization test (Ahmed and Kanwal, 2004). These tests were used to identify the isolate according to the Bergey's manual of systematic bacteriology (Claus and Berkeley, 1986).

Reduction of hexavalent chromium: The ability of the isolated bacterial strain on the reduction of hexavalent chromium was analyzed using potassium dichromate.

The LB broth was prepared with 50 mg/L concentration of $K_2Cr_2O_7$. The medium was inoculated with 2% volume of the test bacteria and incubated at 200 rpm for 5 d. After incubation, the medium was withdrawn and centrifuged to collect the supernatant. The supernatant was analysed for the residual hexavalent chromium (Barlett and James, 1996).

Diphenylcarbazide assay: The amount of hexavalent chromium present in the supernatant was analysed using diphenyl carbazide method described by Alok Prasad Das and Akalabya Bissoyi (2011). The Diphenylcarbazide (DPC) reagent was prepared by dissolving 0.025 g of DPC in 9.67 mL of acetone and 330 μ L of H_2SO_4 . In an Eppendorf tube, 200 μ L of culture supernatant was added. To this 400 μ L of 20 mM MOPS-NaOH buffer, 33 μ L of 3M H_2SO_4 , 40 μ L of DPC reagent and 327 μ L distilled water was added. The absorbance was read immediately at 540 nm.

Optimization studies: The effect of various environmental conditions on the reduction of chromium and bacterial growth was analysed. The impact of inoculum density was studied by inoculating the culture media with a range of 1–5% of bacterial starter. The effects of temperature and incubation time on chromium reduction was studied by conducting the bioprocess at temperature range 25–45°C and incubation period range of 0–72 h at 12 h intervals. The influence of initial pH of medium was studied at the pH range 5–10.

Result and discussion

In this study, a chromium resistant bacterium has been isolated from tannery effluent. The tannery effluent was selected as various researchers reported that the wastewater from the tannery were rich in chromium and chromium reducing bacteria (Basu *et al.*, 1997; Zahoor and Rehman, 2009). It is because the natural habitats of certain bacteria are generally characterized by co-existence of a large number of toxic and nontoxic cations. Of the 7 strains grown on screening medium, the bacterial strain that showed increased growth was selected and it revealed resistance towards the chromium ions in the medium. Chromium is a common pollutant in industrial effluents which contaminates the natural water and its removal has been well documented by various researchers (Pattanapitpaisal *et al.*, 2002; Zahoor and Rehman, 2009). The microbe-catalysed reduction of Cr^{6+} to Cr^{3+} is one of the most potential method which was first reported by Romanenko and Koren'Ken (1977) using *Pseudomonas* sp.

The selected potential bacterial isolate resistant to chromium was subjected to identification by determining its biochemical characteristics as per Bergey's manual of systemic bacteriology. The isolate was found to be gram negative, spore forming, indole negative, MR-VP positive and non-citrate utilizing bacteria. From the biochemical observation, the bacterial isolate was identified as *Bacillus* sp. (data not shown).

Fig. 1. Effect of temperature on chromium degradation by *Bacillus* sp. MRKV.

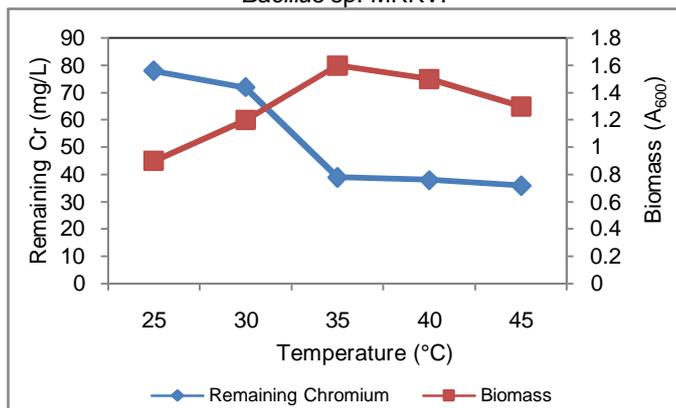


Fig. 2. Effect of pH on chromium degradation by *Bacillus* sp. MRKV.

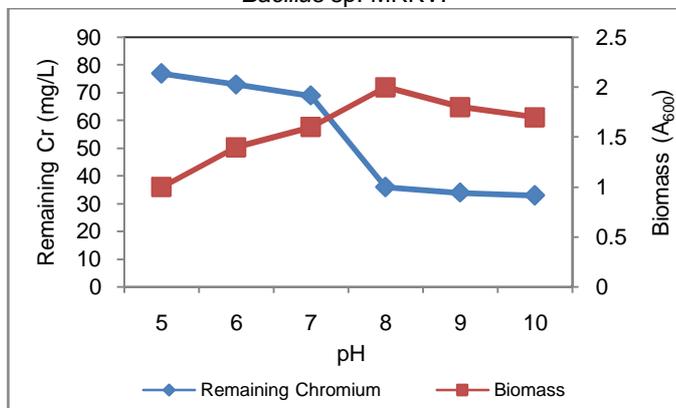
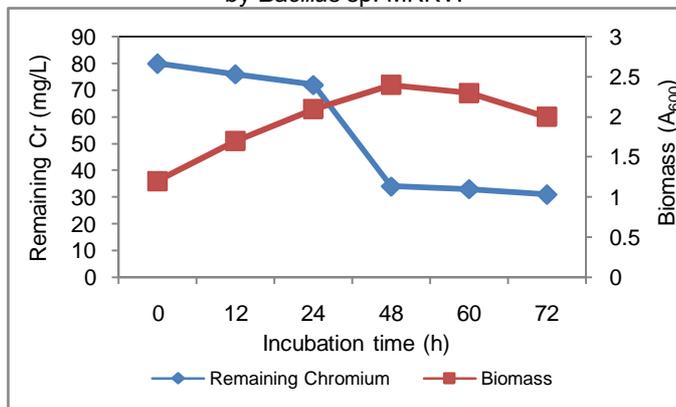


Fig. 3. Effect of incubation time on chromium degradation by *Bacillus* sp. MRKV.



The growth response of bacterial isolate and the reduction of hexavalent chromium ($K_2Cr_2O_7$) were studied in Nutrient broth with respect to different environmental conditions. The optimum incubation temperature for chromium resistant strain on $K_2Cr_2O_7$ amended medium was found to be 35°C (Fig. 1). The isolate also showed maximum growth at pH 8 (Fig. 2). The growth curve pattern was studied by analyzing its growth at different incubation times which elucidates the better growth was obtained during 48th h after inoculation (Fig. 3).

Chromium reducing capability of the bacterial isolates was also assayed simultaneously which revealed that the chromium reduction was directly proportional to the growth of the strain i.e. the rate of chromium reduction increases with respect to the increase in cell density. Some researches including, Farah *et al.* (2010) also suggests the same concept: the rate of increase in cell density is directly proportional to the rate of chromate reduction. The isolate shows merely steady growth throughout the study. This bacterium was able to reduce hexavalent chromium rapidly. Potassium permanganate was selected for the present study as it comes under the category of compounds containing hexavalent chromium (Cr(VI)). Hexavalent chromium is a dangerous carcinogen and oxidizing agent and listed as Class A human carcinogen by the US environmental protection agency (Desai *et al.*, 2008).

Conclusion

This study reported isolation of potent chromium resistant *Bacillus* sp. strain from tannery effluent located in Chennai, India. The strain can effectively reduce hexavalent chromium under wide range of environmental parameters. The isolated bacterial isolate showed excellent activity towards the reduction of chromium. Hence, the bacterial strain *Bacillus* sp. MRKV can be applied in the bioremediation of hexavalent chromium containing wastewaters, as it found to be having potential to reduce the toxic hexavalent chromium to its less toxic trivalent form.

References

- Ahluwalia, S.S. and Goyal, D. 2007. Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresour. Technol.* 98: 2243–2257.
- Ahmed, S. and Kanwal, R. 2004. Biochemical characteristics of lactic acid producing bacteria and preparation of camel milk cheese by using Starter culture. *Pak. Vet. J.* 24(2): 87–91.
- Alok Prasad Das and Akalabya Bissoyi. 2011. Computational approach for comparative phylogenetic analysis of isolated chromium resistant strain *Brevibacterium casei*. *J. Engg. Technol. Res.* 3(3): 82–87.
- Barlett, R.J. and James, B.R. 1996. Chromium. In: *Methods of soil Analysis*, Sparks, D.L. (ed.), SSSA: Madison, WI, pp.683–701.
- Basu, M., Bhattacharya, S. and Paul, A.K. 1997. Isolation and characterization of chromium resistant bacteria from tannery effluents. *Bull. Environ. Contam. Toxicol.* 58: 535–542.
- Chung, J., Nerenberg, R. and Rittmann, B.E. 2006. Bio-reduction of soluble chromate using hydrogen based membrane biofilm reactor. *Water Res.* 40: 1634–1642.
- Claus, D. and Berkeley, R.C.W. 1986. Genus *Pseudomonas*. In: *Bergey's Manual of Systematic Bacteriology*, vol. 1, Sneath, P.H.A., Mair, N.S., Sharpe, M.E. (eds), Baltimore: Williams and Wilkins, pp.140–219.
- Desai, C., Jain, K. and Madamwar, D. 2008. Evaluation of *in vitro* Cr(VI) reduction potential in cytosolic extracts of three indigenous *Bacillus* sp. isolated from Cr(VI) polluted industrial landfill. *Bioresour. Technol.* 99: 6059–6069.
- Farah, R., Shakoori, Shagufta Tabassum, Rehman, A. and Shakoori, A.R. 2010. Isolation and Characterization of Cr⁶⁺ reducing bacteria and their potential use in bioremediation of chromium containing wastewater. *Pak. J. Zool.* 42(6): 651–658.
- Goulhen, F., Gloter, A., Guyot, F. and Bruschi, A. 2006. Cr (VI) detoxification by *Desulfovibrio vulgaris* strain hildenborough: microbe-metal interactions studies. *Appl. Microbiol. Biotechnol.* 71: 892–897.
- Nourbakhsh, M., Sag, Y., Ozer, D., Aksu, Z., Kutsal, T. and Calgar, A. 1994. A comparative study of various biosorbents for removal of chromium (ε) ions from industrial wastewater. *Proc. Biochem.* 29: 1–5.
- Pattanapitpaisal, P., Brown, N.L. and Macaskie, L.E., 2001. Chromate reduction and 16S rRNA identification of bacteria isolated from a Cr (VI)-contaminated site. *Appl. Microbiol. Biotechnol.* 57: 257–261.
- Rehman, A., Zahoor, A., Muneer, B. and Hasnain, S. 2008. Chromium tolerance and reduction potential of a *Bacillus* sp.ev3 isolated from metal contaminated wastewater. *Bull. Environ. Contaminat. Toxicol.* 81: 25–29.
- Romanenko, V.I. and Koren'ken, V.N. 1977. A pure culture of bacteria utilizing chromates and bichromates as hydrogen acceptors in growth under anaerobic conditions. *Mikrobiologiya.* 46: 414–417.
- Stasinakis, A.S., Thomaidis, N.S., Mamais, D., Papanikolaou, E.C., Tsakon, A. and Lekkas, T.D. 2003. Effect of chromium (VI) addition on the activated sludge process. *Water Res.* 37: 2140–2148.
- Thiele, D.J. 1995. Metal detoxification in eukaryotic cells. Crisp Data Base of National Institute of Health: Washington, DC.
- Viamajala, S., Smith, W.A., Sani, R.K., Apel, W.A., Petersen, J.N., Neal, A.L., Roberto, F.F., Newby, D.T. and Peyton, B.M. 2007. Isolation and characterization of Cr (VI) reducing *Cellulomonas* spp. from subsurface soils: implications for long-term chromate reduction. *Bioresour. Technol.* 98: 612–622.
- Wahyudi, A.T., Prasojo, B.J., Mubarik, N.R. 2010. Diversity of antifungal compounds-producing *Bacillus* spp. isolated from rhizosphere of soybean plant based on ARDRA and 16S rRNA. Hayati. *J. Biosci.* 17: 145–150.
- Zahoor, A. and Rehman, A. 2009. Isolation of Cr(VI) reducing bacteria from industrial effluents and their potential use in bioremediation of chromium containing wastewater. *J. Environ. Sci.* 21: 814–820.