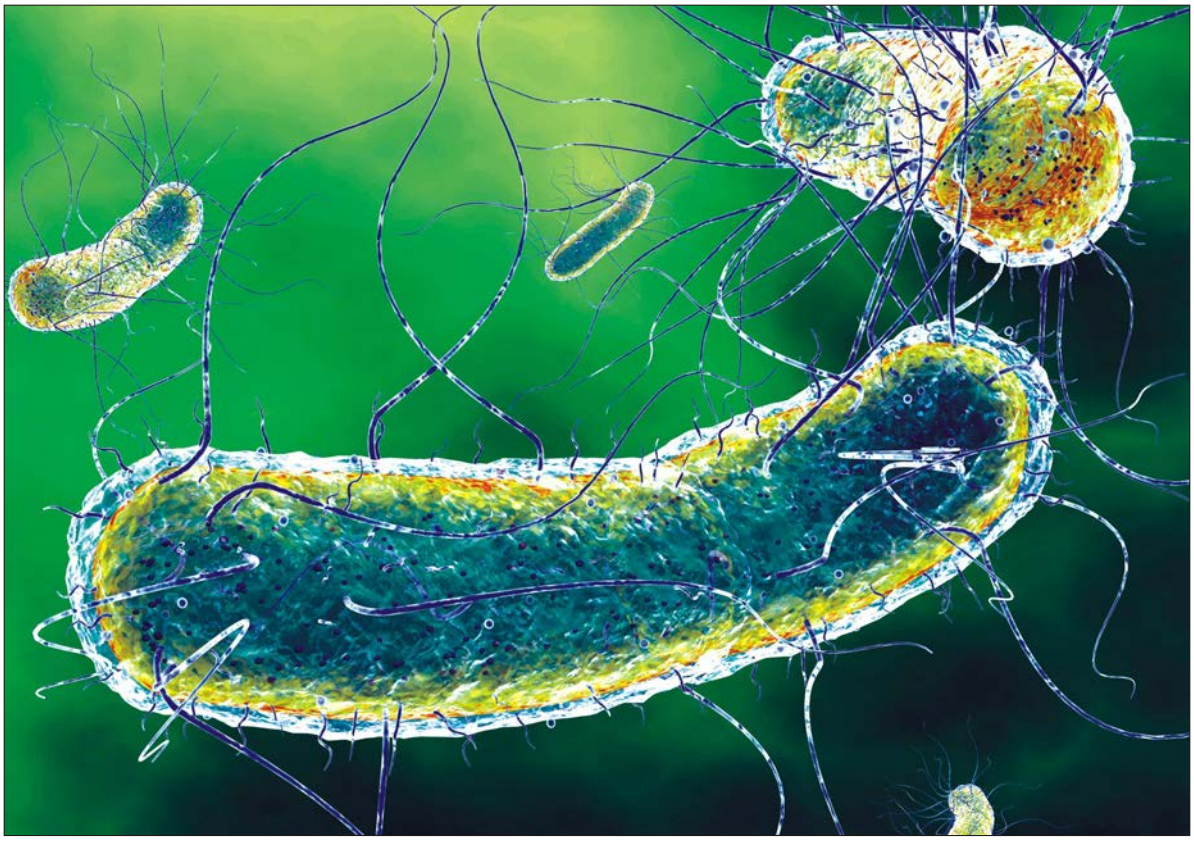

Current Research in Bacteriology

ISSN 1994-5426

science alert

ANSInet
an open access publisher
http://ansinet.com

Current Research in Bacteriology 8 (3): 62-69, 2015
ISSN 1994-5426 / DOI: 10.3923/crb.2015.62.69

CR (VI) Removal by Indigenous *Klebsiella* Species PB6 Isolated from Contaminated Soil under the Influence of Various Factors

Parvaze Ahmad Wani and Adeosun Bukola Omozele
Department of Biological Sciences, College of Natural and Applied Sciences, Crescent University, Abeokuta, Ogun State, Nigeria

Corresponding Author: Parvaze Ahmad Wani, Department of Biological Sciences, College of Natural and Applied Sciences, Crescent University, Abeokuta, Ogun State, Nigeria

ABSTRACT

Chromium (VI) contamination has accelerated due to rapid industrialization worldwide. Aim of this study is to check the bacterial species for their tolerance towards chromium (VI), chromium (VI) reduction under various pH and further check whether these bacteria are reducing Cr (VI) under the influence of various metals, carbon source and protein denaturant. Bacterial strains were isolated from metal contaminated soils of Abeokuta. All of the isolates showed tolerance to chromium (VI). Among all the strains, only *Klebsiella* spp. PB6 showed reduction of chromium (VI). Maximum reduction (89.5%) of chromium (VI) was observed at pH 7 by *Klebsiella* spp. PB6. Similarly, *Klebsiella* spp. PB6 also reduced the chromium considerably at pH 6 (88%) and pH 8 (71%) at a concentration of 100 μg Cr mL^{-1} after 120 h of incubation. *Klebsiella* spp. PB6 also showed chromium (VI) reduction under various metals, protein denaturant and carbon source. There was maximum decrease in Cr (VI) reduction under the influence of $ZnCl_2$ which was followed by $PbCl_2$. Urea and citrate also decreased Cr (VI) reduction compared to control cells. Due to above properties strains could therefore be used as bioremediators of metals in soils contaminated with heavy metals.

Key words: *Klebsiella* species, chromium (VI) tolerance, chromium (VI) reduction, carbon source, metals

INTRODUCTION

The contamination of chromium (VI) is mainly due to the use of Cr (VI) in leather, tanning, metallurgy, electroplating, textile and pigment manufacturing industries (Wang and Xiao, 1995; Pattanapipitpaisal *et al*., 2001a; Sultan and Hasnain, 2007). Chromium occurs either in trivalent or hexavalent which affect growth of microorganisms present in the environment (Ortegel *et al*., 2002). Hexavalent chromium is highly soluble in water, permeable through biological membranes and it interacts with proteins and nucleic acids which makes it more toxic and carcinogenic than trivalent (Kamaludeen *et al*., 2003; Ackerley *et al*., 2006). Reduction of Cr (VI) leads to the formation of stable, less soluble and less toxic Cr (III). Reduction of toxic Cr (VI) to Cr (III) is thus a useful process for remediation of Cr (VI) affected environments (Jeyasingh and Phillip, 2005; Desai *et al*., 2008). The reduction/detoxification of Cr (VI) by microbes is, however, inexpensive and environmentally safe approach and provides a viable option to protect the environment from chromium toxicity. The reduction of Cr (VI) has been reported in *Bacillus* (Elangovan *et al*., 2006; Chaturvedi, 2011), *Pseudomonas* sp., (Wani and Ayoola, 2015; Rahman *et al*., 2007),

Escherichia coli (Bae *et al*., 2005), *Microbacterium* (Pattanapipitpaisal *et al*., 2001b), *Ochrobactrum intermedium* (Faisal and Hansnain, 2005) and *Micrococcus* (Sultan and Hasnain, 2005).

Detoxification of chromium by microbes may occur directly or indirectly and is affected by pH, chromate concentration, incubation periods and the types of microbes involved. In the direct mechanism, microbes enzymatically (chromium reductases) reduce chromium (Losi *et al*., 1994; Dey and Paul, 2013) while in the indirect method, reductants or oxidant, such as H_2S, reduce chromium (DeFilippi and Lupton, 1992). Furthermore, in growing cultures with added carbon sources as electron donors and in cell suspensions, Cr (VI) reduction can be predominantly aerobic or anaerobic but generally not both. Interestingly, chromium reductases can catalyze reduction of Cr (VI) to Cr (III) anaerobically (Lovley and Phillips, 1994), aerobically (Cervantes *et al*., 2001) and also both anaerobically and aerobically (Marsh and Mclnerney, 2001). The Cr (VI) reductase may be present in the membrane fraction of the cells of PGPR, as found in *Pseudomonas fluorescens* and *Enterobacter cloacae* (Wang *et al*., 1990). The reduction to Cr (III) results in the formation of insoluble precipitate [Cr(OH)3], which is easily removed from wastewater (Jeyasingh and Philip, 2005). The enzyme chromium reductase found in *P. ambigua* (Campos-Garcia *et al*., 1997), *Bacillus* sp., (Wang *et al*., 1991) and *Bacillus cereus* Pf-1 (Nguema *et al*., 2014) were purified and characterized. More recently, to clone a chromate reductase gene, novel soluble chromate reductase of *P. putida* was purified to homogeneity and characterized (Puzon *et al*., 2002). The reductase activity was NADH- or NADPH-dependent. Reduction of Cr (VI) by H_2S produced by the bacterial cells is found in soil environments which are rich in sulfate under anaerobic conditions (Losi *et al*., 1994). Hydrogen sulfide, produced in acid sulfate soil under reducing conditions, is easily precipitated as FeS in reduced soils (Eary and Rai, 1991) and sediments. The Fe (II) and H_2S, both microbially produced, are effective reductants of Cr (VI) under reduced conditions as is the FeS (Karnachuk, 1995). Present study was therefore under taken to (1) determine the tolerance pattern of bacteria to Cr (VI), (2) check chromium (VI) reduction by free cells under varying pH (3) check chromium reduction by bacterial cells under the influence of various metals, carbon source and protein denaturant.

MATERIALS AND METHODS

Collection of soil sample: The soil samples for the isolation of chromium (VI) resistant bacteria were collected from the contaminated soils of Abeokuta, Ogun state, Nigeria.

Isolation of bacteria: Bacteria were isolated from the contaminated soil of Abeokuta on nutrient agar medium by spread plate technique. One gram of soil sample was added to a flask containing 9 mL of normal saline solution and was serially diluted. A 10 μL of each suspension was spread plated on solid nutrient agar. Plates were incubated at 28±2°C for 24 h and the bacterial colonies were then purified and preserved on nutrient agar slants for further experiments.

Evaluation of bacterial strains for chromium (VI) tolerance: The isolated bacterial strains were tested for their sensitivity/resistance to chromium (VI) by agar plate dilution method (Holt *et al.,* 1994) using nutrient agar. The freshly prepared agar plates amended with increasing concentration of chromium (0-700 μg mL^{-1}) were spot inoculated (10 μL) with 10^8 cells mL^{-1}. Plates were incubated at 28±2°C for 72 h and the highest concentration of heavy metals supporting growth was defined as the Maximum Resistance Level (MRL). Each experiment was replicated three times.

Chromium (VI) reduction by *Klebsiella* spp: To assess the effect of pH on hexavalent chromium [Cr (VI)] reduction *in vitro*, the Nutrient Broth (NB) was amended with 100 μg mL^{-1} of Cr (VI) and the autoclaved medium was adjusted to pH 5, 6, 7, 8 and 9 with 1 M HCL or 1 M NaOH and incubated at 28±2°C for 120 h. For Cr (VI) reduction, 1 mL culture from each flask was centrifuged (6000 rpm) for 10 min at 10°C and Cr (VI) in the supernatant was determined by 1,5-diphenyl carbazide method (Eaton *et al*., 1992) upto 120 h. Briefly, the test samples were acidified (pH 1-2) and 1,5 diphenyl carbazide (50 μg mL^{-1}) was added and Cr (VI) concentration was detected by UV-VIS spectrophotometer at 540 nm.

Effect of metal ions, electron donors and protein denaturants on Cr (VI) reduction: To assess the effect of (0.1 mM each) metal ions ($CuCl_2$, $ZnCl_2$ and $PbCl_2$), electron donor (citrate) protein denaturants (urea) on hexavalent chromium [Cr (VI)] reduction *in vitro*, the Nutrient Broth (NB) was amended with 100 μg mL^{-1} of Cr (VI) and $CuCl_2$, $ZnCl_2$, $PbCl_2$, citrate and urea. The autoclaved medium was adjusted to pH 7 with 1 M HCL or 1 M NaOH and incubated at 28±2°C for 120 h. After incubation, the bacterial culture solutions were centrifuged (6,000 rpm^{-1} for 20 min at 4°C) and Cr(VI) concentration was measured as described above.

Statistical analysis: Data of three replicates was subjected to statistical analysis using sigma plot 12.0. The values indicate the Mean±SD of three replicates.

RESULTS AND DISCUSSION

Evaluation of bacterial strains for Cr (VI) tolerance: The bacterial strains were evaluated for their tolerance to various concentrations of chromium (VI) using agar plate dilution method (Fig. 1). Generally, bacterial strains showed a varied level of tolerance to Cr (VI). Among the bacterial strains, *Klebsiella* spp. PB6 and *Bacillus* spp. PB 5 showed the highest tolerance to chromium (VI) at a concentration of 1000 μg mL^{-1}. There are reports which have shown the resistance of bacteria to Cr (VI) (Wani and Khan, 2013). There are many reports which have shown varied level of tolerance by bacteria. This varied level of resistance could be due to the variation in growth conditions employed (Rajkumar *et al*., 2005). For example, *Intrasporangium* sp., Q5-1

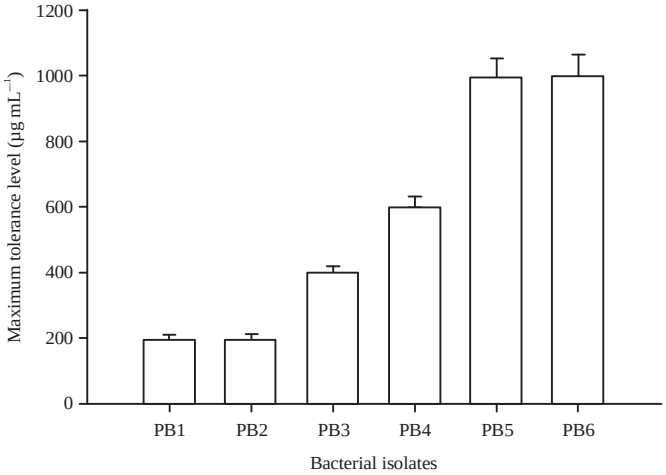

Fig. 1: Maximum tolerance level of different bacterial isolates to chromium (VI)

has shown a tolerance level of 17 mM to Cr (VI) (Yang *et al*., 2009) while as *Bacillus* spp., PZ3 and *Streptococcus* spp. PZ4 showed highest tolerance to chromium (vi) at concentration of 700 μg mL^{-1} (Wani *et al*., 2015). The metal tolerant strains were characterized by physiological, morphological and biochemical characteristics. The strain PB1, PB3, PB4 and PB5 were characterized as *Bacillus* spp. PB2 as *Staphylococcus* spp. while as PB6 was characterized as *Klebsiella* spp.

Effect of pH on Cr (VI) reduction: Chromium is an environmental pollutant released from various industries including tanneries, metal cleaning and processing, chromium plating, wood processing and alloy formation. Chromium [Cr (VI)] is the most toxic and carcinogenic (Kamaludeen *et al*., 2003) due to its high solubility, rapid permeability and their intracellular proteins and nucleic acids (Reeves *et al*., 1983). The reduction of Cr (VI) leads to the formation of stable, less soluble and less toxic Cr (III) and is thus, a useful process for remediation of Cr (VI) affected environments (Thacker *et al*., 2007). Thus, detoxifications of chromium by bacterial strains is a good technique to clean the environment from chromium. Therefore, the present study was designed to determine the Cr (VI) reducing ability of the metal tolerant strains.

Among all the strains only one strain *Klebsiella* spp. PB6 showed chromium reducing ability under *in vitro* conditions as this strain was highly resistant to chromium (VI). This study was carried out to access the effect of different pH values on the reduction of Cr (VI).

The effect of different pH values on the reduction of chromium (VI) is shown in Fig. 2. Maximum reduction (89.6%) of chromium (VI) was observed at pH 7 by *Klebsiella* spp. PB6. Similarly *Klebsiella* spp. PB6 also reduced the chromium considerably at pH 6 (88%) and pH 8 (71%) respectively, at a concentration of 100 μg Cr mL^{-1} after 120 h of incubation.

Our study is in correlation with the study of Yang *et al*. (2009), who also observed considerable reduction of chromium. The Cr (VI) reduction under various pH and different concentrations of Cr (VI) was also observed by Wani *et al*. (2015), who observed maximum reduction of Cr (VI) at pH 7.0 and 100 μg mL^{-1} of chromium. Reduction has also been observed by Soni *et al*. (2013).

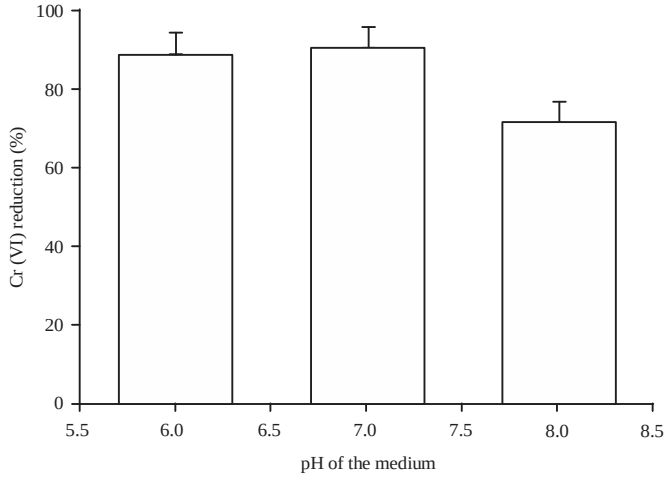

Fig. 2: Effect of pH on Cr (VI) reduction ability of *Klebsiella* spp. PB6 after 120 h of growth in nutrient broth

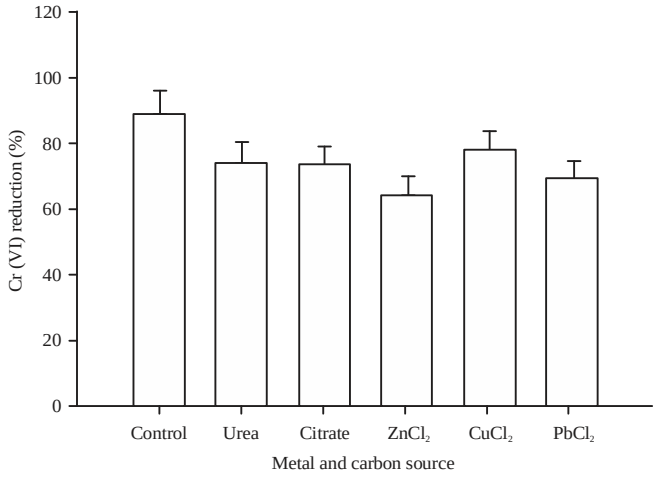

Fig. 3: Effect of carbon, protein denaturant and metals on Cr (VI) reduction by *Klebsiella* spp. PB6 after 120 h of growth in nutrient broth

Effect of metal ions, electron donors and protein denaturants on Cr (VI) reduction: The effect of different metal ions, electron donor and protein denaturant on the reduction of chromium(VI) is shown in Fig. 3. Maximum effect on the reduction by *Klebsiella* spp. PB6 was shown by $ZnCl_2$, followed by $PbCl_2$ whereas, less effect was shown by $CuCl_2$. Citrate and urea also show decrease in chromium reduction but the reduction was still high which was more than 75%. This study has concluded that nutrient broth is best for Cr (VI) reduction in comparison to citrate. Metal ions have been known to affect chromate reductase activity. Ohtake *et al*. (1990) reported that Cu^{2+} inhibit the chromate reductase activity of *Enterobacter cloacae* whereas, Park *et al*. (2000) found reduction in soluble chromate reductase activity in *Pseudomonas putida*. In another study, Pal *et al*. (2013) also found reduction in soluble chromate reductase activity in *B. sphaericus*. Metal ions may affect microbial Cr (VI) reduction in two ways: destruction of cells (decrease in cell growth) and inhibition of enzymes responsible for Cr (VI) reduction. Metal ions may absorb on to cell walls or complex with enzymes responsible for Cr (VI) reduction. Citrate has shown decrease in the Cr (VI) reduction compared to control. It has shown a Cr (VI) reduction of 75% and decreased Cr (VI) reduction by 14.5% compared to control. Similar decrease in Cr (VI) by citrate compared to control has been shown by Soni *et al*. (2013). In another study, Yang *et al*. (2009) found decrease in chromium (VI) reduction by NADH, methanol and ethanol compared to control. In this study, we found decrease in Cr (VI) reduction by acetate possibly because Cr (VI) reductase enzyme of strain PB6 is not acetate dependent. Urea has shown decrease in Cr (VI) reduction compared to control. Similar decrease in Cr (VI) reduction by urea has been studied by Soni *et al*. (2013).

CONCLUSION

Based on the novel properties of chromium resistance and reduction, this bacterial strain could be used for Cr (VI) remediation in chromium contaminated soils.

REFERENCES

Ackerley, D.F., Y. Barak, S.V. Lynch, J. Curtin and A. Matin, 2006. Effect of chromate stress on *Escherichia coli* K-12. J. Bacteriol., 188: 3371-3381.

Bae, W.C., H.K. Lee, Y.C. Choe, D.J. Jahng and S.H. Lee *et al*., 2005. Purification and characterization of NADPH-dependent Cr(VI) reductase from *Escherichia coli* ATCC 33456. J. Microbiol., 43: 21-27.

Campos-Garcia, J., G. Martinez-Cadena, R. Alvarez-Gonzalez and C. Cervantes, 1997. Purification and partial characterization of a chromate reductase from *Bacillus*. Rev. Latinoam. Microbiol., 39: 73-81.

Cervantes, C., J. Campos-Garcia, S. Devars, F. Gutierrez-Corona, H. Loza-Tavera, J.C. Torres-Guzman and R. Moreno-Sanchez, 2001. Interactions of chromium with microorganisms and plants. FEMS Microbiol., 25: 335-347.

Chaturvedi, M.K., 2011. Studies on chromate removal by chromium-resistant *Bacillus* sp. isolated from tannery effluent. J. Environ. Protect., 2: 76-82.

DeFilippi, L.J. and F.S. Lupton, 1992. Bioremediation of soluble Cr (VI) using sulfate reducing bacteria in Allied Signal Research. Proceedings of the National R and B Conference on the Control of Hazardous Materials, San Francisco, February 4, 1992, pp: 1-138.

Desai, C., K. Jain and D. Madamwar, 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.

Dey, S. and A.K. Paul, 2013. Evaluation of *in vitro* reduction of hexavalent chromium by cell-free extract of *Arthrobacter* sp. SUK 1201. Br. Microbiol. Res. J., 3: 325-338.

Eary, L.E. and D. Rai, 1991. Chromate reduction by subsurface soils under acidic conditions. Soil Sci. Soc. Am. J., 55: 676-683.

Eaton, A.D., L.S. Clesceri and A.E. Greenberg, 1992. Standard methods for the examination of water and wastewater. American Public Health Association, American Water Works Association (AWWA), Water Environment Federation (WEF), Washington, DC., USA.

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

Faisal, M. and S. Hasnain, 2005. Bacterial Cr(VI) reduction concurrently improves sunflower (*Helianthus annuus* L.) growth. Biotechnol. Lett., 27: 943-947.

Holt, J.G., N.R. Kreig, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1994. Bergeys Manual of Determinative Bacteriology. 9th Edn., Williams and Wilkins, New York, USA.

Jeyasingh, J. and L. Philip, 2005. Bioremediation of chromium contaminated soil: Optimization of operating parameters under laboratory conditions. J. Hazrad. Mater., 1: 113-120.

Kamaludeen, S.P.B., M. Megharaj, A.L. Juhasz, N. Sethunathan and R. Naidu, 2003. Chromium-microorganism interactions in soils: Remediation implications. Rev. Environ. Contaim. Toxicol., 178: 93-164.

Karnachuk, O.V., 1995. Influence of hexavalent chromium on hydrogen sulfide formation by sulfate-reducing bacteria. Microbiology, 64: 262-265.

Losi, M.E., C. Amrhein and W.T. Frankenberger Jr., 1994. Environmental biochemistry of chromium. Rev. Environ. Contaim. Toxicol., 136: 91-121.

Lovley, D.R. and E.J.P. Phillips, 1994. Reduction of chromate by *Desulfovibrio vulgaris* and its c3 cytochrome. Applied Environ. Microbiol., 60: 726-728.

Marsh, T.L. and M.J. Mclnerney, 2001. Relationship of hydrogen bioavailability to chromate reduction in aquifer sediments. Applied Environ. Microbiol., 67: 1517-1521.

Nguema, P.F., Z. Luo and J.J. Lian, 2014. Enzymatic chromium (VI) reduction by cytoplasmic and cell membrane fractions of chromate-reducing bacterium isolated from sewage treatment plant. Int. J. Biol., 6: 64-76.

Ohtake, H., E. Fujii and K. Toda, 1990. Reduction of toxic chromate in an industrial effluent by use of a chromate reducing strain of *Enterobacter cloacae*. Environ. Technol., 11: 663-668.

Ortegel, J.W., E.D. Staren, L.P. Faber, W.H. Warren and D.P. Braun, 2002. Modulation of tumor-infiltrating lymphocyte cytolytic activity against human non-small cell lung cancer. Lung Cancer, 36: 17-25.

Pal, A., S. Datta and A.K. Paul, 2013. Hexavalent chromium reduction by immobilized cells of *Bacillus sphaericus* AND 303. Braz. Arch. Biol. Technol., 56: 505-512.

Park, C.H., M. Keyhan, B. Wielinga, S. Fendorf and A. Matin, 2000. Purification to homogeneity and characterization of a novel *Pseudomonas putida* chromate reductase. Applied Environ. Microbiol., 66: 1788-1795.

Pattanapipitpaisal, P., N.L. Brown and L.E. Macaskie, 2001a. Chromate reduction by *Microbacterium liquefaciens* immobilised in polyvinyl alcohol. Biotechnol. Lett., 23: 61-65.

Pattanapipitpaisal, P., N.L. Brown and L.F. Macaskie, 2001b. Chromate reduction and 16S rRNA identification of bacteria isolated from a Cr(VI)-contaminated site. Applied Microbiol. Biotechnol., 57: 257-261.

Puzon, G.J., J.N. Petersen, A.G. Roberts, D.M. Kramer and L. Xun, 2002. A bacterial flavin reductase system reduces chromate to a soluble chromium(III)-NAD^+ complex. Biochem. Biophys. Res. Commun., 294: 76-81.

Rahman, M.U., S. Gul and M.Z.U. Haq, 2007. Reduction of chromium (VI) by locally isolated *Pseudomonas* sp. C-171. Turk. J. Biol., 31: 161-166.

Rajkumar, M., R. Nagendran, K.J. Lee and W.H. Lee, 2005. Characterization of a novel Cr^{6+} reducing *Pseudomonas* sp. with plant growth-promoting potential. Curr. Microbiol., 50: 266-271.

Reeves, M.W., L. Pine, J.B. Neilands and A. Balows, 1983. Absence of siderophore activity in *Legionella* species grown in iron-deficient media. J. Bacteriol., 154: 324-329.

Soni, S.K., R. Singh, A. Awasthi, M. Singh and A. Kalra, 2013. *In vitro* Cr(VI) reduction by cell-free extracts of chromate-reducing bacteria isolated from tannery effluent irrigated soil. Environ. Sci. Pollut. Res., 20: 1661-1674.

Sultan, S. and S. Hasnain, 2005. Chromate reduction capability of a gram positive bacterium isolated from effluent of dying industry. Bull. Environ. Contam. Toxicol., 75: 699-706.

Sultan, S. and S. Hasnain, 2007. Reduction of toxic hexavalent chromium by *Ochrobactrum intermedium* strain SDCr-5 stimulated by heavy metals. Biores. Technol., 98: 340-344.

Thacker, U., R. Parikh, Y. Shouche and D. Madamwar, 2007. Reduction of chromate by cell-free extract of *Brucella* sp. isolated from Cr(VI) contaminated sites. Bioresour. Technol., 98: 1541-1547.

Wang, P.C., T. Mori, K. Toda and H. Ohtake, 1990. Membrane-associated chromate reductase activity from *Enterobacter cloacae*. J. Bacteriol., 172: 1670-1672.

Wang, P.C., K. Toda, H. Ohtake, I. Kusaka and I. Yabe, 1991. Membrane-bound respiratory system of *Enterobacter cloacae* strain HO1 grown anaerobically with chromate. FEMS Microbiol. Lett., 78: 11-15.

Wang, Y.T. and C. Xiao, 1995. Factors affecting hexavalent chromium reduction in pure cultures of bacteria. Water Res., 29: 2467-2474.

Wani, P.A. and M.S. Khan, 2013. Nickel detoxification and plant growth promotion by multi metal resistant plant growth promoting *Rhizobium* species RL9. Bullet. Environ. Contam. Toxicol., 91: 117-124.

Wani, P.A. and O.H. Ayoola, 2015. Bioreduction of Cr (VI) by heavy metal resistant *Pseudomonas* species. J. Environ. Sci. Technol., 8: 122-130.

Wani, P.A., I.O. Zainab, I.A. Wasiu and K.O. Jamiu, 2015. Chromium (Vi) reduction by streptococcus species isolated from the industrial area of Abeokuta, Ogun State, Nigeria. Res. J. Microbiol., 10: 66-75.

Yang, J., M. He and G. Wang, 2009. Removal of toxic chromate using free and immobilized Cr(VI)-reducing bacterial cells of *Intrasporangium* sp. Q5-1. World. J. Microbiol. Biotechnol., 25: 1579-1587.