Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 10 (2): 66-75, 2015
ISSN 1816-4935 / DOI: 10.3923/jm.2015.66.75

Chromium (Vi) Reduction by *Streptococcus* Species Isolated from the Industrial Area of Abeokuta, Ogun State, Nigeria

[1]Parvaze Ahmad Wani, [1]Ibrahim Odunola Zainab, [2]Idris Adegbite Wasiu and [1]Kuranga Oluropo Jamiu

[1]Department of Biological Sciences,

[2]Department of Chemical 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 multiple metals, antibiotics and plant growth promoting activity and further check whether these bacteria are reducing Cr (VI). Bacterial strains were isolated from metal contaminated soils of Abeokuta. All of the isolates showed tolerance to lead, zinc and chromium (VI). Bacterial specie also showed tolerance towards antibiotics, 100% of the isolates were tolerant to Septrin, Chloramphenicol, Sparfloxacin, Amoxicillin, Augmentin, Tarivid and Streptomycin, whereas 83.33% were tolerant to Gentamycin and Pefloxacin and 33.33% were resistant to Ciprofloxacin. All bacterial species were positive to ammonia, whereas strain PZ3 and PZ4 were found to be positive to HCN. Among all the strains, only *Streptococcus* spp. PZ4 showed reduction of Chromium (VI). Maximum reduction (85%) of chromium (VI) was observed at pH 7 by *Streptococcus* spp. PZ4. Similarly, *Streptococcus* spp. PZ4 reduced the chromium considerably at pH 5 (51.25%), pH 6 (72.5%), pH 8 (67.5%) and at pH 9 (45%), at a concentration of 100 μg Cr mL^{-1} after 120 h of incubation. *Streptococcus* spp. PZ4 reduced chromium (VI) at a concentration of 50 μg Cr mL^{-1} (47.5%), 100 μg Cr mL^{-1} (91.25%) and 150 μg Cr mL^{-1} (134.17%). Due to above properties strains could therefore be used as bioremediators of metals in soils contaminated with heavy metals and can also increase the yield of various crops under heavy metal contamination.

Key words: *Streptococcus* species, metal tolerance, chromium (VI) reduction, plant growth promoting activities

INTRODUCTION

The contamination of chromium (VI) 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*., 2001; 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 stables, 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).

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* sp. (Elangovan *et al*., 2006; Chaturvedi, 2011), *Pseudomonas* sp. (Rahman *et al*., 2007), *Escherichia coli* (Bae *et al*., 2005), *Microbacterium* (Pattanapipitpaisal *et al*., 2001), *Ochrobactrum intermedium* (Faisal and Hasnain, 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) 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 catalyse reduction of Cr (VI) to Cr (III) anaerobically (Lovley and Coates, 1997), aerobically (Cervantes *et al*., 2001) and also both anaerobically and aerobically (Marsh and McInerney, 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). Further evidence suggested that cytochrome c548 was involved in the reduction of Cr (VI) by membrane vesicles. In the presence of H2 and excess of hydrogenase, cytochrome C3, a periplasmic protein, in the soluble cell free fraction of *D. vulgaris* (Lovley and Coates, 1997), reduced Cr (VI), 50 times faster than did the Cr (VI) reductase of *P. ambigua* with NADH and NADPH, as electron donor (Horitsu *et al*., 1987). Under anaerobic condition Cr (VI) reduction is due to the action of enzymes associated with membranes of the electron transfer system (Cervantes and Campos, 2007). The reduction to Cr (III) results in the formation of insoluble precipitate [$Cr(OH)_3$] which is easily removed from wastewater (Jeyasingh and Phillip, 2005). The enzyme chromium reductase found in *P. ambigua* (Campos-Garcia *et al*., 1997) and *Bacillus* sp. (Wang *et al*., 1991) 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. Fe (II) and H_2S, both microbially produced, are effective reductants of Cr (VI) under reduced conditions as is the FeS (Karnachuk, 1995).

In addition, chromium reducing bacteria also synthesize plant growth promoting substances (Wasi *et al*., 2008). Therefore, the use of bacteria for reduction/detoxification of chromium is one of the preferred choices and is considered as cost effective approach in bioremediation technologies. The present study was therefore under taken (1) To determine the resistance pattern of soil bacteria to heavy metals and antibiotics and (2) To check chromium reduction under varying pH and chromium concentration.

MATERIALS AND METHODS

Collection of soil sample: The soil samples for the isolation of heavy metal resistance microorganisms were collected from the contaminated soils of Abeokuta, Ogun state, Nigeria.

Isolation of bacteria: Bacteria were isolated from the contaminated soils of Abeokuta on nutrient agar medium by spread plate technique. One gram of soil sample was added to a flask containing 100 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 metal tolerance: The isolated bacterial strains from the contaminated soil were tested for their sensitivity/resistance to three heavy metals viz., chromium, zinc and copper 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}), zinc (0-700 μg mL^{-1}) and copper (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.

Determination of antibiotic sensitivity: To determine susceptibility to antibiotics, the bacterial strains were tested for their sensitivity to ten antibiotics. The reactions to antibiotics were determined by the disc diffusion method (Bauer *et al*., 1966). The bacterial strains were grown in nutrient broth at 28±2°C for 24 h. A 0.1 mL of the over-night grown culture was spread on the surface of nutrient agar. The antibiotic discs of known potency were then placed on the agar surface and the plates were incubated at 28±2°C for 24 h. The zones of inhibition around the antibiotic discs were measured and the strains were classified as Resistant (R), Intermediate (I) and Susceptible (S), following the standard antibiotic disc sensitivity testing method (Difco Laboratories, 1984) to the following antibiotics: Septrin (30 μg), Chloramphenicol (30 μg), Ciprofloxacin (10 μg), Sparfloxacin (10 μg), Amoxicillin (30 μg), Augmentin (30 μg), Gentamycin (10 μg), Pefloxacin (30 μg), Streptomycin (30 μg) and Tarivid (10 μg).

***In vitro* assay of hydrogen cyanide and ammonia:** Hydrogen cyanide production by bacterial isolates was detected by the method of Bakker and Schippers (1987). For HCN production, the bacterial strains were grown on an HCN induction medium (30 g tryptic soy broth, 4.4 g glycine and 15 g agar L^{-1}) at 28±2°C for four days. For each bacterial isolate, 100 μL of 10^8 cells mL^{-1} was placed in the centre of the petri plates. A disk of Whatman filter paper No. 1 dipped in 0.5% picric acid and 2% Na_2CO_3 was placed at the lid of the petri plates. Plates were sealed with parafilm. After four days incubation at 28±2°C, an orange brown colour of the paper indicating HCN production was observed.

For ammonia production, the bacterial strains were grown in peptone water (g L^{-1}: peptone 10 g, NaCl 5 g, pH 7) and incubated at 30±2°C for four days. One milliliter of Nessler reagent was added to each tube and the development of yellow color indicating ammonia production was recorded (Dye, 1962).

Chromium (VI) reduction: 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 1M HCl or 1 M NaOH and incubated at 28±2°C for 120 h. Further, to assess the effect of different concentrations (0, 50,100 and 150 μg mL^{-1}) of Cr (VI), the $K_2Cr_2O_7$ were amended in Nutrient broth 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 spectrophotometer (spectronic 20D) at 540 nm.

Data of three replicates were subjected to statistical analysis using pair samples T test with significant level of $p<0.05$. The values indicate the Mean±SD of three replicates.

RESULTS AND DISCUSSION

Evaluation of bacterial for metal tolerance: The bacterial strains were evaluated for their tolerance to various concentrations of chromium (VI) and other metals like zinc and copper using agar plate dilution method (Fig. 1). Generally, bacterial strains showed a varied level of tolerance to heavy metals. Among the bacterial strains, *Bacillus* spp. PZ3 and *Streptococcus* spp. PZ4 showed highest tolerance to Chromium (vi) at concentration 700 µg mL^{-1}, *Pseudomonas* spp. PZ1 and *Streptococcus* spp. PZ2 showed highest tolerance to Copper and Zinc at concentration 700 and 700 µg mL^{-1}, respectively. There are reports which have shown the resistance of bacteria to heavy metals (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, *Rhizobium leguminosarum* have shown a tolerance level of 92.9 µM to zinc (Delorme *et al*., 2003) while *Rhizobium* species isolated from nodules of *Trifolium repense* tolerated 300 mg kg^{-1} nickel and when grown in nickel amended soil could nodulate the legume crop effectively (Smith and Giller, 1992). In the present study, *Bacillus* spp. PZ3 and *Streptococcus* spp. PZ4 showed highest tolerance to Chromium (vi) at a concentration of 700 µg mL^{-1} to each strain, *Pseudomonas* spp. PZ1 and *Streptococcus* spp. PZ2 showed highest tolerance to Copper and Zinc at concentration 700 and 700 µg mL^{-1}, respectively. Bacterial strains showed a high tolerance to chromium which was followed by copper and then zinc. The metal tolerant strains were characterized by physiological, morphological and biochemical characteristics. The strain PZ1 and PZ6 was characterized as *Pseudomonas* spp. PZ2 and PZ4 was characterized as *Streptococcus* spp. PZ3 was characterized as *Bacillus* spp. while PZ5 was characterized as *Micrococcus* spp.

Rhizobium leguminosarum biovar *trifolii* isolated from sewage sludge treated soil showed a high tolerance level of 0.24-0.26 mM to Ni^{2+} and 6.0-8.0 mM to Zn^{2+} (Purchase and Miles, 2001). Similarly, metal tolerance by *Rhizobium*, *Bradyrhizobium* and *Azotobacter* (Pajuelo *et al*., 2008) and varying level of resistance among other PGPR (*Bacillus* and *Pseudomonas*) have also been reported (Yilmaz, 2003; Thacker *et al*., 2007; Wani *et al*., 2008).

Antibiotic resistance of bacterial strains: Resistance to antibiotics varied from one bacterial strain to another strain (Table 1). Among bacterial species, 100% of strains were resistant to

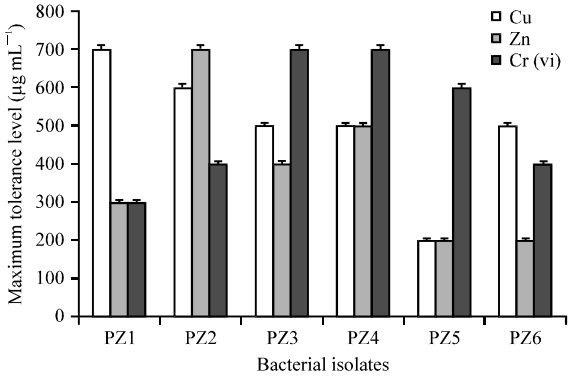

Fig. 1: Maximum tolerance level shown by different bacterial isolates

Table 1: Resistance pattern of bacterial species to various antibiotics

		No. of resistant isolates	
Antibiotics	Concentrations (μg mLG^{1})	No.	%
Septrin	30	6	100.00
Chloramphenicol	30	6	100.00
Sparfloxacin	10	6	100.00
Ciprofloxacin	10	2	33.33
Amoxacillin	30	6	100.00
Augmentin	30	6	100.00
Gentamycin	10	5	83.33
Pefloxacin	30	5	83.33
Tarivid	10	6	100.00
Streptomycin	30	6	100.00

Table 2: Plant growth promoting activities of metal resistant bacterial isolates

	Plant growth promoting activity	
Bacterial isolate No.	Ammonia	HCN
Pseudomonas spp. (PZ1)	+	-
Streptococcus spp. (PZ2)	+	-
Bacillus spp. (PZ3)	+	+
Streptococcus spp. (PZ4)	+	+
Micrococcus spp. (PZ5)	+	-
Pseudomonas spp. (PZ6)	+	-

Septrin, Chloramphenicol, Sparfloxacin, Amoxacillin, Augumentin, Tarivid and Streptomycin, 83.33% were resistant to Gentamycin and Pefloxacin whereas 33.33% were resistant to Ciprofloxacin. Bacterial resistance to antibiotics is an emerging problem these days. Resistance to antibiotics by microbes could be due to change in the genetic makeup, can be due to a genetic mutation or by transfer of antibiotic resistant genes between organisms in the environment (Spain and Alm, 2003). With these considerations, the antibiotic resistance among PGPR was studied which differed from antibiotic to antibiotic for all the PGPR strains. Multi antibiotic resistances shown by the bacterial strains (e.g., *Bacillus* spp. PZ3 and *Pseudomonas* spp. PZ6) may be because of their tolerance to metals which have been reported in many studies (Yilmaz, 2003; Verma *et al*., 2001). It has been observed that metal and antibiotic resistant organisms can adapt faster to metal stress in the environment due to R-factors than by mutation and natural selection (Silver and Misra, 1988). Similar observations on antibiotics resistance by PGPR strains have been reported (Thacker *et al*., 2007). The variation in the resistance to many tested antibacterial drugs (antibiotics) may possibly be due to the differences in growth conditions and exposure of PGPR to stress conditions or toxic substance as well as presence or absence of resistance mechanisms that could be encoded either by chromosome and/or R-plasmid (Spain and Alm, 2003).

***In vitro* assay of HCN and ammonia:** The plant growth promoting rhizobacterial strains were tested further for the synthesis of ammonia and hydrogen cyanide using peptone water and HCN induction medium, respectively (Table 2). Generally, all PGPR strains were found positive for ammonia while PZ3, PZ4 were found to be positive for HCN. In the present study bacterial strains were positive to plant growth promoting activities and produced substantial amount of HCN and Ammonia. The ammonia released by these bacterial strains plays a signaling role in the interaction

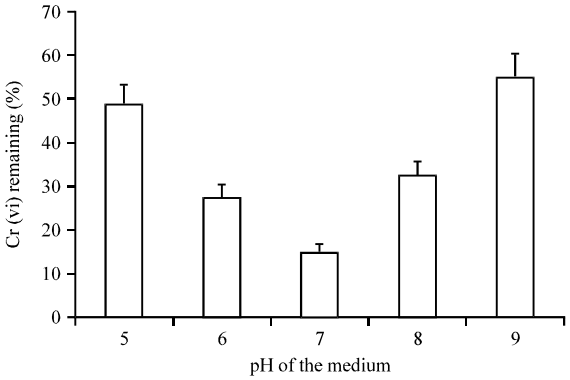

Fig. 2: Effect of pH on Cr (VI) reduction ability of *Streptococcus* spp. PZ4 after 120 h of growth in nutrient broth

between plant growth promoting bacteria and plants (Becker *et al*., 2002). Moreover, the ammonia released by the bacterial strain is known to increase the glutamine synthetase activity (Sood *et al*., 2002). In addition, ammonia transporters found in several PGPR are thought to be involved in the reabsorption of NH_4^+ released as a consequence of NH_3 diffusion through the bacterial membrane (Van Dommelen *et al*., 1997).

Chromium (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 thus 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 *Streptococcus* species PZ4 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 (1) Effect of different pH values on the reduction of Cr (VI) and (2) The effect of chromate concentration on chromium (VI) reduction.

The effect of different pH values on the reduction of chromium (VI) is shown in Fig. 2. Maximum reduction (85%) of chromium (VI) was observed at pH 7 by *Streptococcus* spp. PZ4. Similarly, PGPR isolates *Streptococcus* spp. PZ4 reduced the chromium considerably at pH 5 (51.25), pH 6 (72.5%), pH 8 (67.5%) and at pH 9 (45%), respectively, at a concentration of 100 µg Cr mL^{-1} after 120 h of incubation.

In this study, the chromium reducing ability of PGPR strain was assessed using nutrient broth supplemented with 50, 100 and 150 µg mL^{-1} of $K_2Cr_2O_7$ in order to determine the effect of chromium (VI) reducing ability of the selected culture under *in vitro* conditions (Fig. 3). The time for total reduction of chromium (VI) increased with increase in the concentration of chromium (VI). During this study, the complete reduction of chromium (VI) occurred after 120 h by *Streptococcus* spp. PZ4 (Fig. 2) at 50 µg mL^{-1} of chromium. The PGPR strain *Streptococcus* spp. PZ4

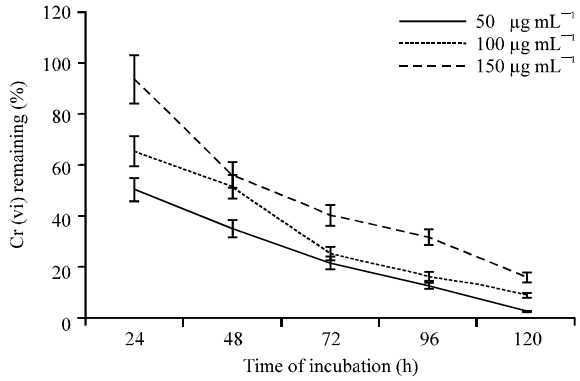

Fig. 3: Effect of Cr (VI) on Cr (VI) reduction ability of *Streptococcus* spp. PZ4 in nutrient broth after 120 h of incubation

reduced chromium (VI) at concentration of 50 µg Cr mL^{-1} (47.5%), 100 µg Cr mL^{-1} (91.25%) and 150 µg Cr mL^{-1} (134.1%), respectively. Our study is in correlation with the study of Yang *et al*. (2009) who also observed considerable reduction of chromium.

CONCLUSION

This study concludes that the bacterial strains not only tolerated heavy metals, antibiotics, produced plant growth promoting substances but also reduced Chromium (VI) under different pH and chromium concentration. Due to multifarious properties expressed by the bacterial strains, these strains could therefore be used as bioremediators of metals in soils contaminated with heavy metals and can also increase the yield of various crops under heavy metal contamination.

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.

Bakker, A.W. and B. Schippers, 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* SPP-mediated plant growth-stimulation. Soil Biol. Biochem., 19: 451-457.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Becker, D., R. Stanke, I. Fendrik, W.B. Frommer, J. Vanderleyden, W.M. Kaiser and R. Hedrich, 2002. Expression of the NH_4^+-transporter gene *LEAMT1;2* is induced in tomato roots upon association with N_2-fixing bacteria. Planta, 215: 424-429.

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

Cervantes, C. and G.J. Campos, 2007. Reduction and Efflux of Chromate by Bacteria. In: Molecular Microbiology of Heavy Metals, Nies, D.H. and S. Silver, (Eds.). Springer, Berlin, Germany, ISBN-13: 978-3540697701, pp: 407-420.

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.

Delorme, T.A., J.V. Gagliardi, J.S. Angle, P.B. van Berkum and R.L. Chaney, 2003. Phenotypic and genetic diversity of rhizobia isolated from nodules of clover grown in a zinc and cadmium contaminated soil. Soil Sci. Soc. Am. J., 67: 1746-1754.

Difco Laboratories, 1984. Difco Manual: Dehydrated Culture Media and Reagents for Microbiology. 10th Edn., Difco Laboratories Inc., Detroit, MI., USA.

Dye, D.W., 1962. The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. N. Z. J. Sci., 5: 393-416.

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. APHA/AWWA/WEF., Washington, DC., Pages: 981.

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.

Horitsu, H., S. Futo, Y. Miyaazawa, S. Ogai and K. Kawai, 1987. Enzymatic reduction of hexavalent chromium by hexavalent chromium tolerant *Pseudomonas ambigua* G-1. Agric. Biol. Chem., 47: 2417-2420.

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. Jr. Frankenberger, 1994. Environmental biochemistry of chromium. Rev. Environ. Contaim. Toxicol., 136: 91-121.

Lovley, D.R. and J.D. Coates, 1997. Bioremediation of metal contamination. Curr. Opin. Biotechnol., 8: 285-289.

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

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.

Pajuelo, E., I.D. Rodriguez-Llorente, M. Dary and A.J. Palomares, 2008. Toxic effects of arsenic on *Sinorhizobium-Medicago sativa* symbiotic interaction. Environ. Pollut., 154: 203-211.

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

Purchase, D. and R.J. Miles, 2001. Survival and nodulating ability of indigenous and inoculated *Rhizobium leguminosarum* biovar *trifolii* in sterilized and unsterilized soil treated with sewage sludge. Curr. Microbiol., 42: 59-64.

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.

Silver, S. and K.T. Misra, 1988. Plasmid-mediated heavy metal resistances. Annu. Res. Microbiol., 42: 717-743.

Smith, S.R. and K.E. Giller, 1992. Effective *Rhizobium leguminosarum* biovar Trifolii present in five soils contaminated with heavy metals from long-term applications of sewage sludge or metal mine spoil. Soil Biol. Biochem., 24: 781-788.

Sood, C.R., S.V. Chanda and Y.D. Singh, 2002. Effect of different nitrogen sources and plant growth regulators on glutamine synthetase and glutamate synthase activities of radish cotyledons. Bulg. J. Plant Physiol., 28: 46-56.

Spain, A. and E. Alm, 2003. Implications of microbial heavy metal tolerance in the environment. Rev. Undergraduate Res., 2: 1-6.

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.

Van Dommelen, A., E. van Bastelaere, V. Keijers and J. Vanderleyden, 1997. Genetics of *Azospirillum brasilense* with respect to ammonium transport, sugar uptake and chemotaxis. Plant Soil, 194: 155-160.

Verma, A., K. Kukreja, D.V. Pathak, S. Suneja and N. Narula, 2001. *In vitro* production of plant growth regulators (PGRs) by *Azotobacter chroococcum*. Indian J. Microbiol., 41: 305-307.

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., M.S. Khan and A. Zaidi, 2008. Chromium reducing and plant growth promoting *Mesorhizobium* improves chickpea growth in chromium amended soil. Biotechnol. Lett., 30: 159-163.

Wasi, S., G. Jeelani and M. Ahmad, 2008. Biochemical characterization of a multiple heavy metal, pesticides and phenol resistant *Pseudomonas fluorescens* strain. Chemosphere, 71: 1348-1355.

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.

Yilmaz, E.I., 2003. Metal tolerance and biosorption capacity of *Bacillus circulans* strain EB1. Res. Microbiol., 154: 409-415.