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Differential Responses of Marine Sediment Bacteria *Pseudomonas stutzeri* Strain VKM014 to Chromate Exposures

[1]P.V. Bramhachari, [2]J. Ravichand, [1]K.V. Deepika, [1]P. Yalamanda and [3]K.V. Chaitanya
[1]Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, India
[2]Department of Biotechnology, Goa University, Goa, India
[3]Department of Biotechnology, GIT, GITAM University, Vishakhapatnam, India

Corresponding Author: P.V. Bramhachari, Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh, 521 001, India

ABSTRACT

The aim of the present study was to isolate and characterize the bacterial strain of a chromium-contaminated site and to evaluate the differentially expressed proteins of strain VKM014, when exposed to varied concentrations of heavy metal chromium. Sediment samples were collected from the Port of Kakinada situated on the Southern Part of East Coast of India and plated on Nutrient agar medium containing chromate. Strain VKM014 produced both mucoid biofilm and biosurfactant. The strain VKM014 which could tolerate high levels of chromate (0.75 mM) was further selected for strain characterization, growth kinetics and expression of chromate-induced proteins. Based on morphological, biochemical characteristics and phenogram, the isolate was tentatively grouped under *Pseudomonas* sp. and identified as *Pseudomonas stutzeri* based on 16S rRNA gene sequence analysis (GenBank EF079450). This study results indicated that chromate upshock resulted in induction/enhancement of two unidentified 25 and 28 kDa induced proteins in the cell extract of the VKM014 following the exposures to 0.2 and 0.5 mM of chromate. In contrast, changes at biochemical level (induced proteins) are the first detectable responses to environmental perturbations and so the sensitive indicators of pollution. This study demonstrates new links between biosurfactant production, differential subpopulation response and metal exposures and involvement of certain enzymes in chromate resistance.

Key words: Chromium, *Pseudomonas stutzeri*, phenogram, growth kinetics, chromate-induced proteins

INTRODUCTION

Cr(VI) (chromate) is a serious environmental pollutant due to the extensive use of chromium compounds in variety of industrial activities such as chrome leather tanning, metal cleaning and processing, wood preservation, alloy preparation, corrosion control, electroplating, pigment manufacture and shipping activities has led to the release of this toxic metal into the environment in large quantities (Barceloux, 1999; Sultan and Hasnain, 2000). Chromium occurs in oxidation states Cr(0) to Cr(VI) but only Cr(III) and Cr(VI) are biologically significant, interconvertible depending on pH, inorganic ions and organic matter content (Avudainayagam *et al*., 2006; Apte *et al*., 2005). Characteristics like higher solubility in water, rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acid makes

hexavalent form of Cr comparatively more toxic than trivalent one (Basu *et al*., 1997). Trivalent chromium, Cr(III) is an essential micronutrient for humans and is relatively less soluble (Bahijri and Mufti, 2002) than the hexavalent chromium where as the high valence chromium is toxic (Lock and Janssen, 2002), mutagenic (Feng *et al*., 2004; Gohar and Mohammadi, 2010). The chromate resistance mechanisms displayed by microorganisms are diverse and include biosorption, intracellular accumulation either through direct obstruction of the ion uptake system or active chromate efflux, precipitation and reduction of Cr(VI) to less toxic Cr(III) (Cervantes *et al*., 2001). Few reports examining the molecular effects and global changes in protein expression due to chromate stress on *Shewanella oneidensis* (Chourey *et al*., 2006) and *E. coli* (Ackerley *et al*., 2006) are available. However, it is interesting to note that so far there are no reports on up regulation of chromate-induced stress proteins from the marine sediment bacteria. The objective of this study was to isolate and characterize the bacterial strain of a chromium-contaminated site and to evaluate the differentially expressed proteins of *Pseudomonas stutzeri* strain VKM014, when exposed to varied concentrations of heavy metal chromium.

MATERIALS AND METHODS

Sampling and isolation of bacterial strains: Sampling site chosen for this study was the Port of Kakinada situated on the Southern Part of East Coast of India at latitude 16.59' (North) and Longitude 82.19'. (East). A metal-contaminated sediment samples were collected from the vicinity of ships and surrounding ship-building areas (0-25 cm depth) using a Niskin sampler, in sterile polycarbonate bottles kept at 4°C and used within two days of collection. Water samples were mechanically shaken prior to use and allowed to stand for 10 min to permit settling of heavy particles. A volume of 0.1 mL of water sample was placed on Nutrient Agar (NA) amended with 0.5 mM Chromate.

Identification of *Pseudomonas stutzeri* strain VKM014: Genomic DNA was prepared from 5 mL late-exponential-phase cells. Genomic DNA was resuspended in 50 μL TE buffer (10 mM tris-HCl, 1 mM EDTA, pH 8.0) and stored at -20°C until 16S rRNA genes were amplified. The resistant strain were designated as VKM014 and identified as *Pseudomonas stutzeri* with 16S rRNA sequencing with primers F'341-CCTACGGGAGGC AGCAG and reverse R' 1387-GCCCGGGAACGTATTCACCG) and submitted to GenBank to get the accession No. EF079450. The Polymerase Chain Reaction (PCR) mixture consists of 20 μL PCR buffer $MgCl_2$ (25 mM), dNTP (2.5 mM), (10 μM) each of the primer, 0.5 μL Taq Polymerase and 50 ng of template DNA. The DNA was amplified using PCR machine with conditions: denaturation at 94°C, 3 min, 35 cycles consisting of 94°C for 30 sec, 55°C for 60 sec, 72°C for 90 sec and final extension at 72°C for 10 min.

Qualitative detection of mucoid biofilm-producing bacteria: Qualitative detection of biofilm formation was studied by culturing the strains on Congo Red Agar (CRA) plates as described previously (Jain and Agarwal, 2009). The media contained TSB broth (37 g L^{-1}), sucrose (0.8 g L^{-1}), agar (10 g L^{-1}) and Congo red stain (0.8 g L^{-1}). The Congo red stain was prepared as a concentrated aqueous solution, autoclaved separately and added to the media after the agar had cooled to 55°C. CRA plates were inoculated with all the isolates and incubated aerobically for 24 h at 37°C. After incubation, pigmented colonies (generally black colour) were considered as mucoid phenotype positive.

Biosurfactant production: Determination of biosurfactant production was done in accordance with Maier and Soberon-Chavez (2000). Biosurfactant production was determined by the reduction in surface tension in comparison to an uninoculated control.

Cr(VI) tolerance tests: In present laboratory the cells were maintained as a batch culture in a standard medium recommended for *Pseudomonas* species (Girard and Snell, 1983) at a temperature of 28°C with constant shaking. Culture growth was monitored by measuring optical density at 600 nm. The viability was detected by cell growth on agar plates with a cell suspension dilution. Overnight cultures were used as inoculum, which were incubated in basal mineral medium (without Cr(VI)) containing glucose (1.0 g L^{-1}) at 30°C with shaking. The number of viable cells in the inoculum varied between 6.3×10^7 and 5×10^8 bacteria mL^{-1} and 1 mL was used to inoculate the experiments.

Polyacrylamide gel electrophoresis of whole cell proteins: The protein profiles were analyzed for the potential induction of chromate stress proteins. Cells were harvested by centrifugation at 10,000 rpm for 10 min (4°C) and washed twice in 0.15 M NaCl. The cell pellets were treated with 500 μL of lysis buffer pH 7.5. The cells were suspended in ice- cold deionized water were sonicated by linear probe (44 kHz) for 5×1 min bursts (1 min interval between each burst) on ice using a sonicator Cells debris was removed by centrifugation at 3,000 rpm for 20 min (4°C) and cell wall was recovered by centrifugation at 15,000 rpm for 20 min (4°C). Ten microliters of the filtrates were loaded on a SDS-PAGE (12%) and electrophoresed using tris-glycine-SDS buffer followed by silver staining and documentation of the gel (Apte and Bhagwat, 1989).

RESULTS AND DISCUSSION

The Port of Kakinada is one of the principal sea ports amongst the minor ports in India and one among the biggest shipyards of east coast of India and hence a potential source of chromate contamination. The selected strain VKM014 was tentatively identified as *Pseudomonas* sp. based on Gram staining, cell morphology by using optical microscopy and classical biochemical tests following Bergey's Manual of Systematic Bacteriology. The PCR-amplified 16S rRNA of chromate resistant isolate VKM014 was sequenced and sequence homologies were analyzed using BLAST search of the NCBI. The 16S sequence database was used to create phylogenetic tree of the studied isolate. The complete sequences were aligned using multiprocessor of CLUSTALW programmed at European Bioinformatics site. Aligned sequences were used for phylogenetic tree with neighbor joining methods to assess topology. The analysis was performed in programmed package (MEGA, Version-4) with default parameters of Kimura-2 model. Figure 1 shows the phylogenetic relationships of one out-group taxa of *E. coli* strain U00096 and 9 related taxa pertaining to chromate resistant bacteria based on a combined sequence analysis of partial 16S rDNA genes was inferred using the neighbor-joining method (Tamura *et al*., 2007).

Phenotypic mucoid biofilm production was assessed by culturing the isolated strain on Congo red agar plates. The strain VKM104 grown on CRA medium was characterized by black colonies as depicted in Fig. 2. This mucoid morphotype also produced a good quantity of an unidentified biosurfactant as measured by the ability of the supernatant to reduce the surface tension of the medium. The large morphotype reduced surface tension of the medium from 62 to 40 dyn cm^{-1}, a relatively good reduction in surface tension (Maier and Soberon-Chavez, 2000).

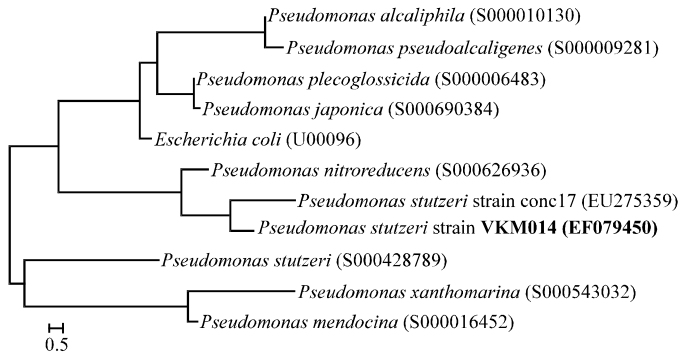

Fig. 1: Phylogenetic relationships of chromate resistant bacteria *Pseudomonas stutzeri* strain VKM014 (bold), one out-group taxa, *E. coli* strain U00096 and 9 related taxa pertaining to chromate resistant bacteria based on a combined sequence analysis of partial 16S rDNA genes. The tested strain in the present study is highlighted in bold and with GenBank accession numbers in parentheses. The tree was constructed from a matrix of pairwise genetic distances using the neighbor-joining tree method using standard parameters of MEGA version 4.0

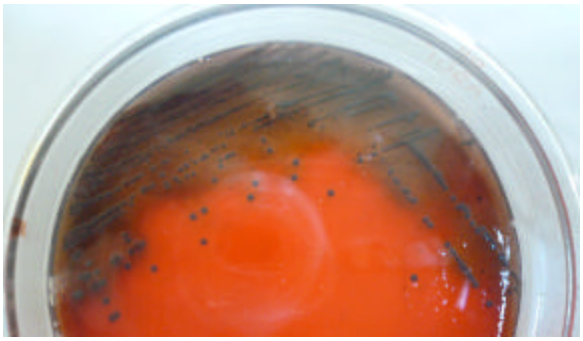

Fig. 2: Mucoid biofilm production by *Pseudomonas stutzeri* strain VKM014, which denotes defined black colonies on Congo red agar plates

The marine sediment *Pseudomonas stutzeri* strain VKM014 (EF079450), which could tolerate high levels of chromate (i.e., 0.75 mM), was selected for strain characterization, growth kinetics and expression of chromate-induced proteins. The culture was initially subjected to 0.2-0.5 mM of chromate, following the treatments, isolate VKM014 was able to grow in the presence of chromate up to 1 mM. To determine the difference in lag time, a time course study comparing the growth and protein profiles in medium with chromate and without chromate was performed. Growth of *P. stutzeri* VKM014 was pronouncedly inhibited above 0.75 mM chromate. The extended lag time in the presence of chromate suggests the cells changed physiologically as a result of the stressful chromate environment. Recent evidences obtained by Dogan *et al.* (2011) and Christl *et al.* (2012) represented *Pseudomonas* sp. that can potentially be applied for in situ bioremediation of Cr(VI) contaminated sites. However, other researchers have observed these bacterial strains *Serratia* sp. Cr-10 (Zhang and Li, 2011), *Flexivirga alba* ST13T (Sugiyama *et al.*, 2011), *Bacillus* sp.

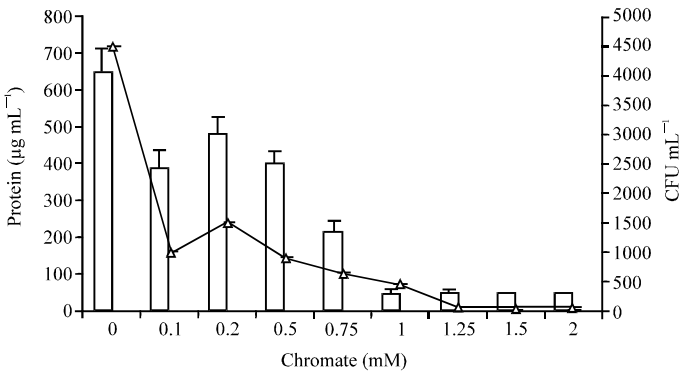

Fig. 3: Survival of *Pseudomonas stutzeri* strain VKM014 at different concentrations of chromate

(Faisal and Hasnain, 2006; Qazilbash *et al*., 2006; Kannan *et al*., 2007; Masood and Malik, 2011), *Halomonas* sp. TA-04 (Focardi *et al*., 2012) exhibited chromate resistance in bacteria from chromate-contaminated sites, tannery effluents dosed with chromate (Tripathi and Garg, 2010). On the contrary, antibiotic resistant bacteria that are exposed to chromium were also reported (Saxena *et al*., 2006).

These results demonstrated the induction of a stress-protein from *strain* VKM014, when this bacterium was exposed to specified concentrations of chromate. The comparative graphical analysis in Fig. 3 showed that the protein content was markedly decreased from the control when exposed to 0.1 mM chromate, though it increased with 0.2 and 0.5 mM chromate, the subsequent increase in concentration decreases the protein content up to 1 mM of chromate. The protein composition during long-term exposure to Cr(VI) at 36 h, which led to the formation of insoluble Cr(III) on the bacterial surface, accompanied by changes in total protein composition (Fig. 4). An impoverished protein composition in the presence of 0.2 and 0.5 mM chromate and induction of stress proteins was evidently observed on the whole cell protein analysis on SDS PAGE. However, it should be noted that cells under long-term Cr(VI) exposure i.e., 36 h in presence of 0.75-1 mM the chromate shock inhibited the synthesis of some proteins (Fig. 4). Induction of 25, 28, 32, 36, 38, 48 and 56 kDa proteins were specific and a significant enhancement of the proteins was observed in response to the chromate up shock. The 25 and 28 kDa proteins in *Pseudomonas stutzeri* strain VKM014 was different from earlier observations, which is expressed in presence of 0.2 and 0.5 mM chromate. The probable reason could be that the level of chromate which is optimum for growth and induction of metallothionein like polypeptides. So at that concentration the 25 and 28 kDa proteins gets induced, while the proteins above 32 kDa, which are present in all the conditions gets up-regulated, might be constitutively expressed. In contrast, any changes at biochemical level (i.e., induced proteins) are the considered the first detectable responses to serious environmental perturbations and so the sensitive indicators of pollution. Similar reports demonstrated the exposure of *Pseudomonas aeruginosa* to chromium resulted in differential expression of cellular proteins (Khare *et al*., 1997). This comparable up-regulation of proteins have also been mentioned earlier in TBTCl resistant *Vibrio* sp., which exhibited enhanced synthesis of two polypeptides of 30 and 12 kDa when cells grown in presence of 125 µM TBTC (Fukagawa *et al*., 1992). In this study, we report that exposure of a single population of cells to chromate resulted in a differential growth response and production of unidentified metallothionein like small molecular weight proteins induced between 14.3 and 66 kDa a complex array of responses to chromium toxicity.

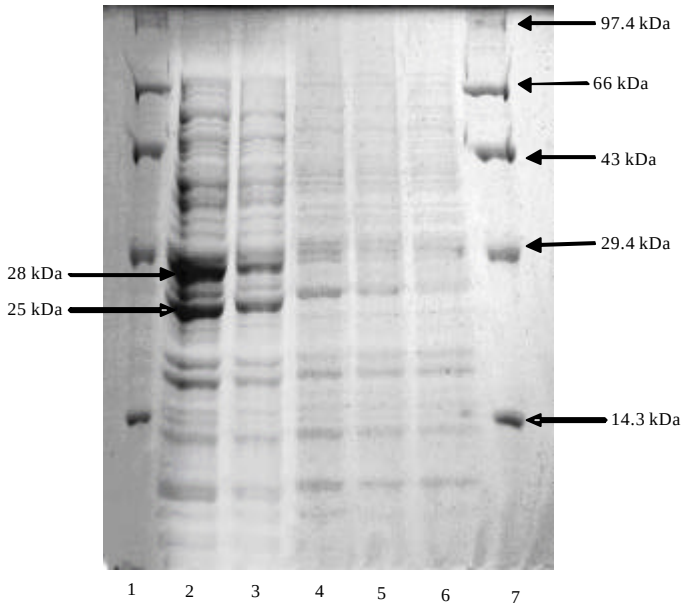

Fig. 4: Protein profile of *Pseudomonas stutzeri* strain VKM014 exposed to chromate metal. Lane 1: Medium range protein molecular weight marker, Lane 2: Whole cell protein of cells grown for 36 h with 0.2 mM K_2CrO_4, Lane 3: Whole cell protein of cells grown for 36 h with 0.5 mM K_2CrO_4, Lane 4: Whole cell protein of cells grown for 36 h with 0.75 mM K_2CrO_4 Lane 5: Whole cell protein of cells grown for 36 h with 1 mM K_2CrO_4, Lane 6: Whole cell protein of cells grown without K_2CrO_4, Lane 7: Medium range protein molecular weight marker, The induced protein is located between the 14.3 and 66 kDa molecular weight marker

The heavy metal induced specific polypeptides play an imperative role in metal ion homeostasis in cyanobacteria (Olafson *et al*., 1979). Some bacterial strains are also known to synthesize cysteine rich low molecular weight polypeptides which play an important role in biosorption of these metals ultimately resulting in immobilization of toxic metals thereby protecting their vital metabolic process catalyzed by enzymes (Neidhardt and van Bogelen, 1987; Barrios *et al*., 1994). These proteins alleviate or reduce damage to the cell by acting as chaperones that bind to and stabilize unfolded or nascent proteins and as carrier molecules (Barrios *et al*., 1994).

Certain heavy metal tolerant bacteria such as *Escherichia coli*, *Shewanella*, *Alcaligenes*, *Pseudomonas*, *Bacillus* and *Vibrio* sp. and numerous species exhibited metal induced synthesis of low molecular weight, cysteine rich polypeptides (metallothioneins) which bind with specific metals such as cadmium and copper making them unavailable to the bacterial cells (Cervantes and Silver, 1992; Gadd, 2004). The site of action of chromate may be both at the cytoplasmic membrane as well as intracellular level. Studies on the effect of chromate metal ion on certain microbial enzymes indicates that in some bacteria, chromate can interact with cytosolic enzymes such as Glutathione Reductase (GR), cysteine, carbohydrates and nucleotides) NAD (P) H-dependent flavoprotein family of reductase, different specific and nonspecific membrane-associated reductase (Ishibashi *et al*., 1990; Cervantes and Silver, 1992; Codd *et al*., 2006; Iftikhar *et al*., 2007). This

transition metal also acts on bacterial cell wall (teichoic acids, polycarbohydrates and other diol-containing substances (Seltmann and Holst, 2001). These studies have confirmed that even toxic compounds could affect protein synthesis as reported earlier that a 60 and 40 kDa acid-soluble cell wall proteins were constitutively expressed by *Arthrobacter oxydans* which can detoxify chromium, either by reduction or accumulation (Abuladze *et al*., 2002; Asatiani *et al*., 2004). Several chromate reductases have been identified in diverse bacterial species. Whereas, soluble Cr(VI)-reducing enzymes have been purified to varying degrees from *P. putida* PRS2000 (Ishibashi *et al*., 1990) and *Pseudomonas putida* MK1 (Park *et al*., 2000). Chromium stress changed the protein profile of *Pseudomonas stutzeri* strain VKM014, which indicated that chromium stress might regulate the expression of some genes. A chromate stress induced protein ChrA is reported to be responsible for an energy dependent chromium efflux system in *Pseudomonas*, *Cupriavidus* and *Alcaligenes* sp. (Cervantes and Silver, 1992; Nies, 1999; Park *et al*., 2000) and their possible involvement in resistance/reducing of chromate Cr(VI) to Cr(III).

CONCLUSION

In summary, the exposure of a single population of *Pseudomonas* sp. VKM104 cells to chromium resulted in an inverted growth response, differential subpopulation production of surfactant and production of 2 unidentified 25 and 28 kDa induced proteins protein-a complex array of responses to chromium toxicity. It is hypothesized that exposure to a threshold level of chromium stress (>1 mM) causes individual cells of *Pseudomonas* sp. VKM104 to increase production of surfactant. Increased levels of surfactant production may protect cells in the population by complexing chromium and reducing the bioavailable concentration of chromium. The genes encoding chromate-induced polypeptides can be used to construct a chromate biosensor to directly monitor chromate levels in the industrial effluents. Therefore, the success of microbial-based metal remediation technologies requires a better understanding of the microbial community and the population response to metal stress. Further work is in progress on the gene (s) encoding chromate induced proteins.

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REFERENCES

Abuladze, M.K., N.V. Asatiani, N.G. Bakradze, T.M. Kartvelishvili and H.Y. Holman *et al*., 2002. Effect of chromium action on the protein composition of *Arthrobacter oxydans*. Fresenius Environ. Bull., 11: 562-567.

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.

Apte, A.D., S. Verma, V. Tare and P. Bose, 2005. Oxidation of Cr(III) in tannery sludge to Cr(VI): Field observations and theoretical assessment. J. Hazard. Mater., 121: 215-222.

Apte, S.K. and A.A. Bhagwat, 1989. Salinity-stress induced proteins in two nitrogen fixing Anabena strains differentially tolerant to salt. J. Bacteriol., 171: 909-915.

Asatiani, N.V., M.K. Abuladze, T.M. Kartvelishvili, N.G. Bakradze and N.A. Sapojnikova *et al.*, 2004. Effect of chromium (VI) action on *Arthrobacter oxydans*. Curr. Microbiol., 49: 321-326.

Avudainayagam, S., M. Megharaj, G. Owens, R.S. Kookana, D. Chittleborough and R. Naidu, 2006. Chemistry of chromium in soils with emphasis on tannery waste sites. Rev. Environ. Contam. Toxicol., 178: 53-91.

Bahijri, S.M.A. and A.M.B. Mufti, 2002. Beneficial effects of chromium in people with type 2 diabetes and urinary chromium response to glucose load as a possible indicator of status. Biol. Trace Element Res., 85: 97-110.

Barceloux, D.G., 1999. Chromium. J. Toxicol. Clin. Toxicol., 37: 173-194.

Barrios, C., C. Georgopoulos, P.H. Lambert and G. Del Giudice, 1994. Heat shock proteins as carrier molecules: *In vivo* helper effect mediated by *Escherichia coli* GroEL and DnaK proteins requires cross-linking with antigen. Clin. Exp. Immunol., 98: 229-233.

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

Cervantes, C. and S. Silver, 1992. Plasmid chromate resistance and chromate reduction. Plasmid, 27: 65-71.

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

Chourey, K., M.R. Thompson, J. Morrell-Falvey, N.C. ver Berkmoes and S.D. Brown *et al.*, 2006. Global molecular and morphological effects of 24 h chromium (VI) exposure on *Shewanella oneidensis* MR-1. Applied Environ. Microbiol., 72: 6331-6344.

Christl, I., M. Imseng, E. Tatti, J. Frommer, C. Viti, L. Giovannetti and R. Kretzschmar, 2012. Aerobic reduction of chromium(VI) by *Pseudomonas corrugata* 28: Influence of metabolism and fate of reduced chromium. Geomicrobiol. J., 29: 173-185.

Codd, R., P. Lay, N.Y. Tsibakhashvili, T.L. Kalabegishvili, I.G. Murusidze and H.Y.N. Holman, 2006. Chromium(V) complexes generated in *Arthrobacter oxydans* by simulation analysis of EPR spectra. J. Inorg. Biochem., 100: 1827-1833.

Dogan, N.M., C. Kantar, S. Gulcan, C.J. Dodge, B.C. Yilmaz, M.A. Mazmanci and M.A. Mazmanci, 2011. Chromium (VI) bioremoval by *Pseudomonas* bacteria: Role of microbial exudates for natural attenuation and biotreatment of Cr(VI) contamination. Environ. Sci. Technol., 45: 2278-2285.

Faisal, M. and S. Hasnain, 2006. Detoxification of Cr(VI) by *Bacillus cereus* S-6. Res. J. Microbiol., 1: 45-50.

Feng, Z., W. Hu, W.N. Rom, M. Costa and M.S. Tang, 2004. Chromium (VI) exposure enhances polycyclic aromatic hydrocarbon-DNA binding at the p53 gene in human lung cells. Carcinogenesis, 24: 771-778.

Focardi, S., M. Pepi, G. Landi, S. Gasperini, M. Ruta, P. Di Biasio and S.E. Focardi, 2012. Hexavalent chromium reduction by whole cells and cell free extract of the moderate halophilic bacterial strain *Halomonas* sp. TA-04. Int. Biodeterior. Biodegrad., 66: 63-70.

Fukagawa, T., S. Suzuki, K. Fukagawa, T. Suzuki and K. Takama, 1992. Isolation characterization of tributyltin chloride resistant marine *Vibrio*. FEMS Microbiol. Lett., 93: 83-86.

Gadd, G.M., 2004. Microbial influence on metal mobility and application for bioremediation. Geoderma, 122: 109-119.

Girard, B. and E. Snell, 1983. Biochemical Factors. In: Manual of Methods for General Bacteriology, Gerhardt, P. (Ed.). Mir Publisher, Moscow, Russia, pp: 198-276.

Gohar, A.V. and A. Mohammadi, 2010. Epigenetic effects of carcinogens. J. Biol. Sci., 10: 200-208.

Iftikhar, S., M. Faisal and S. Hasnain, 2007. Cytosolic reduction of Toxic Cr(VI) by Microorganism. Res. J. Environ. Sci., 1: 77-81.

Ishibashi, Y., C. Cervantes and S. Silver, 1990. Chromium reduction in *Pseudomonas putida*. Applied Environ. Microbiol., 56: 2268-2270.

Jain, A. and A. Agarwal, 2009. Biofilm production, a marker of pathogenic potential of colonizing and commensal *Staphylococci*. J. Microbiol. Methods, 76: 88-92.

Kannan, S. K., K.J. Lee , R. Krishnamoorthy , A. Purusothaman , K. Shanthi and N.R. Rao, 2007. Aerobic chromate reducing *Bacillus cereus* isolated from the heavy metal contaminated ennore creek sediment, North of Chennai, Tamil Nadu, South East India. Res. J. Microbiol., 2: 133-140.

Khare, S., A. Ganguli and A.K. Tripathi, 1997. Responses of *Pseudomonas aeruginosa* to chromium stress. Eur. J. Soil Biol., 33: 153-158.

Lock, K. and C.R. Janssen, 2002. Ecotoxicity of chromium (III) to *Eisenia fetida, Enchytraeus albidus* and *Folsomia candida*. Ecotoxicol. Environ. Safety, 51: 203-205.

Maier, R.M. and G. Soberon-Chavez, 2000. *Pseudomonas aeruginosa* rhamnolipids: Biosynthesis and potential applications. Applied Microbiol. Biotechnol., 54: 625-633.

Masood, F. and A. Malik, 2011. Hexavalent chromium reduction by *Bacillus* sp. strain FM1 isolated from heavy-metal contaminated soil. Bull. Environ. Contam. Toxicol., 86: 114-119.

Neidhardt, F.C. and R.A. van Bogelen, 1987. Heat Shock Response. In: *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology, Neidhart, F.C. (Ed.). Vol. 2, American Society for Microbiology, Washington, DC., ISBN-13:9780914826897, pp: 1334-1345.

Nies, D.H., 1999. Microbial heavy-metal resistance. Applied Microbiol. Biotechnol., 51: 730-750.

Olafson, R.W., K. Abel and R.G. Sim, 1979. Prokaryotic metallothionein: Preliminary characterization of a blue-green alga heavy metal-binding protein. Biochem. Biophys. Res. Commun., 89: 36-43.

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.

Qazilbash, A.A., R. Faryal, K.B. Naqvi, S. Ahmad and A. Hameed, 2006. Efficacy of indigenous *Bacillus* species in the removal of chromium from industrial effluent. Biotechnology, 5: 12-20.

Saxena, M.P., P. Kaur, H.M. Saxena and J. Kapur-Ghai, 2006. Antibiotic resistant bacteria isolated from fish died on exposure to chromium. J. Fish. Aquatic Sci., 1: 209-212.

Seltmann, G. and O. Holst, 2001. The Bacterial Cell Wall. Springer, Berlin, Heidelberg, New York.

Sugiyama, T., H. Sugito, K. Mamiya, Y. Suzuki, K. Ando and T. Ohnuki, 2011. Hexavalent chromium reduction by an actinobacterium *Flexivirga alba* ST13T in the family dermacoccaceae. J. Biosci. Bioeng., 113: 367-371.

Sultan, S. and S. Hasnain, 2000. Hexavalent chromium resistant bacteria from effluents of electroplating: Isolation, characterization and chromium (VI) reduction potential. Pak. J. Biol. Sci., 3: 450-456.

Tamura, K., J. Dudley, M. Nei and S. Kumar, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol., 24: 1596-1599.

Tripathi, M. and S.K. Garg, 2010. Studies on selection of efficient bacterial strain simultaneously tolerant to hexavalent chromium and pentachlorophenol isolated from treated tannery effluent. Res. J. Microbiol., 5: 707-716.

Zhang, K. and F. Li, 2011. Isolation and characterization of a chromium-resistant bacterium *Serratia* sp. Cr-10 from a chromate-contaminated site. Applied Microbiol Biotechnol., 90: 1163-1169.