Journal of Applied & Environmental Microbiology, 2018, Vol. 6, No. 3, 67-72
Available online at http://pubs.sciepub.com/jaem/6/3/2

DOI:10.12691/jaem-6-3-2

Genetic Characterization of *Pseudomonas stutzeri* Strain M15-10-3, the Highly Efficient Cr Accumulator Isolated from Leather Tanning Industrial Wastewater

Alawiah Mohammad Alhebshi[1], Ebtesam El-Bestawy[2,*]

[1]Department of Biological Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia
[2]Department of Environmental Studies, Alexandria University, Alexandria, Egypt
*Corresponding author: ebtesamelbestawy@yahoo.com

Received October 02, 2018; Revised November 12, 2018; Accepted December 04, 2018

Abstract The main objective of the present research is to identify and characterize the essential genes responsible for Cr removal by the highly resistant bacterial strain ***Pseudomonas stutzeri*** Strain M15-10-3 isolated from leather tanning industrial wastewater to optimize its capacity and to be exploited in the development of chromium bioaccumulation capacity in other microorganisms. ***Pseudomonas stutzeri*** (**PS**) was previously investigated among a total of 20 bacterial isolates (17 indigenous and 3 exogenous) to decontaminate heavily polluted leather tannery wastewater using batch and fixed (biofilm) mode where it exhibited remarkable efficiency in removing Cr, the highly toxic and main contaminant in tannery effluent. **PS** and 3 sub strains of ***Bacillus cereus*** **ATCC 14579** were subjected to 4 elevated Cr levels (2000-5000 mg/l) for 9 days to confirm the **PS** affinity for Cr bioaccumulation. Results proved the highest affinity of **PS** (≈ 80%) to bioaccumulate Cr from polluted media even at very high concentration (5000 mg/l). Moreover, Cr has remarkable inhibition activity on the growth of ***Bacillus cereus*** strains (75 to 99.7%) even at the lowest tested Cr concentration without any stimulation at all the tested concentrations. However, **PS** exhibited the superior acclimatization ability against Cr even at the highest Cr concentration reflecting its high Cr resistance with 25% growth stimulation at 4000 mg Cr/l and the lowest growth inhibition (37.1% at 2000 mg Cr/l). Therefore, **PS** was considered highly efficient candidate for Cr removal and was selected to be molecularly investigated to characterize Cr resistance genes. Results demonstrated that ChrT gene responsible for chromate reduction ability was most probably present in ***Pseudomonas stutzeri*** genome.

Keywords: *bioaccumulation, chromium, genetic characterization, leather tanning industry, Pseudomonas stutzeri Strain M15-10-3, wastewater*

Cite This Article: Alawiah Mohammad Alhebshi, and Ebtesam El-Bestawy, "Genetic Characterization of *Pseudomonas stutzeri* Strain M15-10-3, the Highly Efficient Cr Accumulator Isolated from Leather Tanning Industrial Wastewater." *Journal of Applied & Environmental Microbiology*, vol. 6, no. 3 (2018): 67-72. doi: 10.12691/jaem-6-3-2.

1. Introduction

As a result of industrialization, metal pollutants such as chromium, mercury, lead, zinc, uranium and selenium are introduced to the environment posing a real threat to the human race [1]. Among heavy metals, chromium (Cr) is described as a highly toxic metal that severely affects human health as well as the surrounding environment [2,3]. The harmful health effects of chromium ions include skin allergy, vomiting, lung and nervous system damage, severe diarrhea and human hemorrhage. Moreover, it possess mutagenic and carcinogenic properties [4,5]. Chromium enters the environment through wastes of many different industries such as mining and refining raw materials, leather tanning, industrial and household sludge, fly ash from holocausts, radioactive materials, pesticides or preservatives and metal plating [6,7,8]. Therefore, it is a must to remove, in particular, Cr^{6+}, from drinking and wastewater due to its high toxicity.

There are several chemical methods for the removal of toxic metals such as solvent extraction, ion exchange, chemical precipitation, membrane separation process, oxidation-reduction, filtration, adsorption, incineration as well as electrochemical treatment [9,10]. However, such methods have many disadvantages among which the generation of toxic sediment or waste products which in turn require safe disposal [11]. Many microorganisms (viable and dead; free or fixed) proved to act as biological remediants and absorbers to remove heavy metals from wastewater [12] especially the highly toxic such as Cr^{6+}, thus, considered as viable, eco-friendly and cost effective technology for cleanup of chromium (VI). Chromium detoxification includes transformation of a highly toxic, mutagenic and carcinogenic hexavalent Cr^{6+} into its non-toxic reduced trivalent form Cr^{3+}. Recently, two soluble Cr^{6+} reductases, ChrR and YieF, have been

isolated from *Pseudomonas putida* MK1 and *Escherichia coli*, correspondingly. The direct use of Cr^{6+} reductases may be a favorable way for bioremediation of wide range of Cr^{6+}- contaminated environments [13].

Pseudomonas aeruginosa Rb-1 and *Ochrobactrum intermedium* Rb-2 enhanced wheat seed germination under chromium (III and IV) stress compared with non-bacterial inoculated control [14]. Strains Rb-1 and Rb-2 that are able to survive in chromium-contaminated environment could improve wheat growth along with decreasing the toxicity of chromate by different direct and indirect mechanisms [15]. Not only bacteria, but viable cells of the white rot fungus *Phanerochaete chrysosporium* (MTCC787) was evaluated to remediate chromium from fortified solution where it removed 99.7% Cr (VI) after 72 h and considered highly potential for decontaminated polluted media [16].

2. Materials & Methods

2.1. Microorganisms and Culturing Media

Pseudomonas stutzeri Strain M15-10-3 (PS) isolated from leather tanning industrial wastewater, and for comparison, 3 sub strains of *Bacillus cereus ATCC* 14579, a well-known metals bio-accumulator Gram +ve bacteria were investigated for Cr bioaccumulation to confirm PS activity. *Bacillus cereus* strains were isolated from pesticide contaminated - agricultural soil. Selected strains were grown in nutrient agar (NA) or broth (NB) supplied by Oxoid LTD (Basingstoke, Hampshire, England) as dehydrated media. Prior to each experiment, cultures were reactivated overnight.

2.2. Cr - Bioaccumulation Assay

The four selected isolates were activated from 24-h agar cultures in 100 ml NB. Cultures were incubated for 24 h at 37 °C to obtain dense inocula. Four sets of four 250 ml flasks (16 flasks total) were prepared with 100 ml sterilized NB. Each set was emended with 4 elevated Cr levels (2000-5000 mg/l final concentration). Total viable count of bacteria (TVC) of the 24 h - liquid culture of each strain was determined after appropriate serial dilution and divided into 4 aliquots (25 ml) and added under aseptic conditions to the Cr- amended NB in the four 250 ml flasks. After inoculation, all cultures were incubated at 37°C. After 9 exposure days 6 ml sample from each culture were aseptically drawn for TVC and Cr residuals determination.

2.3. Total Viable Count of Bacteria (TBVC)

TBVC (CFU/ml) of the selected strains before and after exposure to Cr were serially diluted (up to 10^{-8}), and 1000 µl of the appropriate dilution was cultured (3 replica each) under aseptic conditions on NA plates and incubated for 24 h at 37°C using the pour plate technique of the standard heterotrophic plate count method [17]. Colony forming units (CFU) of the total viable bacterial counts (TVC) were recorded (Colony Counter Stuart Colony Counter Protected by Bio Cote) and averages were calculated.

2.4. Chromium Determination

Chromium levels were determined in the stock solution as well as in the broth cultures amended with Cr before and after treatment with the selected bacteria using Atomic Absorption Spectrophotometer, ASS {Thermo Scientific, S SERIES, AA Spectrophotometer, England, (European Union)}. After incubation, 5 ml of each culture was aseptically drawn, centrifuged (6000 rpm) for 10 min, bacterial pellets was discarded and Cr residues in the broth were determined using the AAS. Removal efficiency of Cr by the selected bacteria was calculated to determine the effectiveness of the remediation process according to the following equation:

$$Removal\ Efficiency\left(RE\ \%\right) = C0 - RC / C0\ X\ 100$$

Where C0 = Initial Concentration before Treatment (Zero Time);
RC= Residual Concentration after Treatment at each Exposure Time.

2.5. Extraction and Purification of Total DNA from Cr-Resistant PS

Extraction of total DNA was carried out using bacterial DNA extraction kit; GeneJET Genomic DNA Purification Kit (Thermo scientific co., Molecular biology, EU). DNA was extracted and purified for PCR amplification by the kit protocol.

2.6. PCR Detection of Cr Accumulating Genes

PCR reactions were carried out using specific primers for Cr resistance [18]. The PCR amplifications were performed in a final volume of 25 µl with 2x MyTaq Red Mix PCR Master Mix (Bioline Co.), according to the manufacturer's instructions. The reaction mixtures included 0.5µl (10 PM) of each of the specific primers and 1µl template DNA [19]. Two pairs of primers, Scfmn1-forward (F), Scfmn1-reverse (R), Scfmn- forward (F) and Scfmn -reverse (R) are shown in Table 1 (Biosearch technologies Co., USA), were designed and used to amplify the fragment of the *Pseudomonas stutzeri* gene (*ChrT*) which concerning to Cr reduction [18]. The full-length *ChrT* gene was obtained using specific primers (Scfmn-F and Scfmn-R) corresponding to the 5'-and 3'-ends of the *ChrT* gene. Amplification has been performed with thermal Cycler (TECHNE, TC-3000) using the following program: denaturation for 1 min at 95 °C; then 35 cycles consisting of 95 °C for 30 sec, annealing temperatures of 54 °C for 30 sec and 72 °C for 1 min; as well as a final extension step at 72 °C for 5 min.

Table 1. Primers used in the study, Procedure Primers Primer sequences (5'-3')

ChrT gene fragment	
Scfmn1-F	GATGTGCAACAAGACGAAGGT
Scfmn1-R	GATGACTCCGCCCATAAACTC
Full-length ChrT gene	
Scfmn-F	ATCATGTCAGATACCTTGAAAGTGG
Scfmn-R	TGCTTTAACCCGCCGAATATA

F, forward; R, reverse.

The PCR product was purified by Gene JET Gel extraction Kit (Thermo scientific Co., Fermentas, EU) after slicing the specific band from the loaded gel. The purified PCR product was stored in -20°C for further need of sequencing.

2.7. Identification of the Amplified DNA Fragments

PCR amplification products were analyzed using electrophoresis in 1 % (w/v) agarose (Sigma) gel and run in 1X TBE buffer, pH 8.3 [20]. The ethidium bromide stained gels to visualize DNA bands, and gels were photographed using transmitted UV in a gel documentation system (Syngene Ingenius). Gene size was determined by comparison with a DNA ladder (100 bp DNA ladder, Solis BioDyne Co.) [21].

3. Results

3.1. Bioaccumulation Assay of Cr by the Efficient Strains

In a comparative study, PS and 3 sub strains of *Bacillus cereus* ATCC 14579 were subjected to 4 elevated Cr levels (2000-5000 mg/l) for 9 days to confirm the PS affinity and superiority for Cr bioaccumulation. Table 2 represents comparison between *Pseudomonas stutzeri* strain M15-10-3 (PS) and *Bacillus cereus* ATCC 14579 sub strains for the removal efficiencies of Cr.

Results revealed general unexpected trend by all the tested strains where they exhibited +ve relation between Cr concentrations and its RE. In that respect, Cr removal by all strains increased with increasing the concentration reaching their highest RE at 5000 mg/l.

Removal efficiency (RE%) of Cr by the selected strains confirmed the superiority of *Pseudomonas stutzeri* strain M15-10-3 compared to the other tested strains where it exhibited the highest RE% at 2000 and 4000 mg Cr/l (70.7 and 77.81% respectively). It almost achieved similar REs at 3000 and 5000 mg Cr/l as *Bacillus cereus* ATCC 14579 (23). *Bacillus cereus* ATCC 14579 strain 13 showed the lowest RE of Cr at all the tested concentrations (58.9-69.72%) while *Bacillus cereus* ATCC 14579 strain 12 showed intermediate RE% of Cr. These results proved the highest affinity PS to bioaccumulate Cr from polluted media even at very high concentration (5000 mg/l) and for wide range. Therefore, PS was selected to be molecularly investigated to determine Cr resistance genes.

3.2. Effect of Chromium on the Growth of the Selected Bacteria

As shown in Table 3 Cr has remarkable inhibition activity on the growth of almost all the selected bacteria except PS. Inhibition% for *Bacillus cereus* strains ranged from 75 to 99.7% even at the lowest tested Cr concentration. None of them exhibited any stimulation at all the tested concentrations. However, PS exhibited superior acclimatization ability against Cr even at the highest Cr concentration reflecting its high Cr resistance. Moreover, it showed 25% growth stimulation at 4000 mg Cr/l which considered marvelous ability. Compared with *Bacillus cereus* strains, PS showed the lowest growth inhibition (37.1%) at 2000 mg Cr/l and almost similar inhibition at 3000 and 5000 mg Cr/l. Therefore, PS is considered highly efficient candidate for Cr removal. Studying PS genes responsible for this ability is of high importance not only for optimizing this ability but also to be exploited in the development of chromium bioaccumulation capacity in other microorganisms for the ultimate target of decontaminating polluted media.

Table 2. Comparison among *Pseudomonas stutzeri* strain M15-10-3 (PS) and *Bacillus cereus* ATCC 14579 Sub Strains for Cr Removal

Strain	Cr (mg/l)							
	2000		3000		4000		5000	
	RC	RE%	RC	RE%	RC	RE%	RC	RE%
P. stutzeri M15-10-3	586.1	70.7**	877.2	70.8	887.7	77.81**	1035.3	79.3**
B. cereus ATCC 14579 (12)	607.7	69.6	873.4	70. 9	979.6	75.51	1193.3	76.1
B. cereus ATCC 14579 (13)	822.1	58.9*	1107.3	63.1*	1211.2	69.72*	1523.2	69.5*
B. cereus ATCC 14579 (23)	626.4	68.7	794.6	73.5**	945.4	76.36	943.4	81.1**

RC: Residual Concentration (mg/l), RE: Removal Efficiency (%), * Lowest RE%,
** Highest RE%, Results are averages of 3 replica.

Table 3. Inhibition or Stimulation % of Total Viable Count (TVC) of *Pseudomonas stutzeri* strain M15-10-3 (PS) and *Bacillus cereus* ATCC 14579 Sub Strains after Exposure to Cr

Strain	Raw Culture	TVC (CFU/ml)							
		2000 mg Cr/l		3000 mg Cr/l		4000 mg Cr/l		5000 mg Cr/l	
		Count	GI% or GS%	Count	GI% or GS%	Count	GI% or GS%	Count	GI% or GS%
Pseudomonas stutzeri M15-10-3	$10.5x10^7$	$66x10^6$	37.1*	$2x10^6$	98.0*	$1.4x10^8$	25.0**	$1.5x10^7$	85.7*
Bacillus cereus ATCC 14579 (12)	$4x10^7$	$9x10^6$	77.5*	$10.5x10^5$	97.4*	$5x10^6$	87.5*	$10x10^6$	75.0*
Bacillus cereus ATCC 14579 (13)	$5.1x10^7$	$15x10^4$	99.7*	$1x10^6$	98.0*	$4x10^6$	92.2*	$2x10^6$	96.1*
Bacillus cereus ATCC 14579 (23)	$6x10^7$	$1x10^6$	98.3*	$1x10^6$	98.3*	$7x10^6$	88.3*	$3x10^6$	95.0*

*GI: Growth Inhibition, **GS: Growth Stimulation, Results are Averages of 3 Replica.

3.3. Identification of Cr-Resistant Genes in PS

The ChrT gene encodes a chromate reductase enzyme which catalyzes the reduction of Cr (VI). The chromate reductase is also known as flavin mononucleotide (FMN) reductase (FMN_red). Firstly, two pairs of specific primers were synthesized on the basis of the FMN red gene sequence of *Serratia* sp. AS13. When the genomic DNA from the *Pseudomonas stutzeri* cells was used as a template, an expected 305-bp fragment of the ChrT gene was amplified using the Scfmn1-F/R primers, which was subsequently sequenced. Then, a full-length ChrT gene of 400 bp and 650 bp were obtained with the specific Scfmn-F/R primers (Figure 1). This result demonstrated that the ChrT gene was most probably present in *Pseudomonas stutzeri* genome which is specific gene responsible for chromate reduction ability [18].

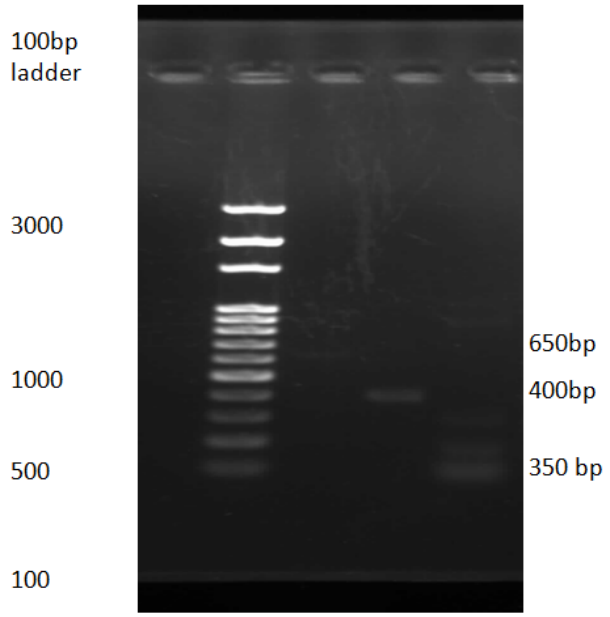

Figure 1. Agarose Gel electrophoreses (1 %) showing PCR amplification of the ChrT gene: Lane 1, DNA marker; lane 4, PCR amplification of the ChrT gene fragment; a 305-bp fragment obtained by PCR amplification with the Scfmn1-F and Scfmn1-R primers, lane2 and 3, PCR amplification of the complete DNA; the 400-bp and 650bp ChrT gene obtained by PCR amplification with the Scfmn-F and Scfmn-R primers

4. Discussion

Among heavy metals, chromate Cr(VI) is a toxic, soluble environmental contaminant. Bacteria can reduce chromate to the insoluble and less toxic Cr(III), thus, leads to effective bioremediation. As mentioned above, many microorganisms (bacteria, fungi, yeast….etc.) can efficiently act as biosorbents for heavy metals from contaminated media either in viable or dead form and either as free or fixed forms [12,13,14,15,16]. Cr especially Cr^{6+} considered highly toxic posing both acute and chronic threats on the different compartments of the environment, thus, it is a must to remove, in particular, Cr^{6+}, from drinking and wastewater. Microbial reduction of Cr^{6+} to Cr^{3+} is well known and effective mechanism to reduce Cr toxicity and an important step in its removal from contaminated media. Among bacteria, various genera were found highly capable of reducing Cr (VI) including *Arthrobacter* [22], *Bacillus* [23], *Microbacterium* [24], *Brucella* [25] and *Pseudomonas* [13,14,15,26]. This highly supports the present study where *P. stutzeri* and *Bacillus cereus* exhibited superior ability for Cr removal (79.3 and 81.1% respectively) at 5000 mg Cr/l due to the included genes.

Pseudomonas spp., in particular, characterize by effective biodegradation and bioaccumulation capability towards wide range of environmental organic and inorganic pollutants including heavy metals. *P. stutzeri* strain M15-10-3 examined in the present study was selected based on its ability to achieve more than 98% Cr from tannery effluent with an initial concentration of 3500 mg/l [27,28]. Generally, many researches are concerned with isolation and identification of naturally occurring microorganisms from contaminated environments with Cr removal capability [29], while few have focused on genes and proteins responsible for Cr reduction [30]. The direct use of Cr^{6+} reductases such as ChrR and YieF, isolated from *Pseudomonas putida* MK1 and *Escherichia coli* may be a favorable way for bioremediation of wide range of Cr^{6+}-contaminated environments [13]. Moreover, *Pseudomonas aeruginosa* Rb-1 and *Ochrobactrum intermedium* Rb-2 enhanced wheat seed germination under chromium (III and IV) stress compared with non-bacterial inoculated control [14]. They could decrease chromate toxicity by different direct and indirect mechanisms [15].

Genetic and protein engineering of suitable enzymes improve bacterial bioremediation. The ChrT gene encodes a chromate reductase enzyme which catalyzes the reduction of Cr (VI). The chromate reductase is also known as flavin mononucleotide (FMN) reductase (FMN_red) [18]. This gene was in the *Serratia* sp. strain S2 and was successfully constructed in other bacteria using genetic engineering technology [31]. The engineered strain, which contained the chromate reductase ChrT gene from *Serratia* sp. S2, was studied for its Cr (VI) reduction efficiency, optimal culture conditions and chromate reductase activity in metal-contaminated soil and water ecosystems. It could achieve up to 40% Cr (VI) reduction rate of at a concentration of 50 mg/l after 48 h exposure with optimal culture conditions of pH 7.0, 37˚C. and chromate reductase reached 14.83 U/mg [32].

Many bacterial enzymes can reduce Cr(VI) to Cr(V), generating excessive reactive oxygen species (ROS) and making those enzymes not appropriate for bioremediation, as they harm the bacteria and do not produce Cr(III) as their primary end product. Thus, not every Cr reductase enzyme is suitable for Cr reduction. For example, pure soluble bacterial flavoproteins; ChrR (from *Pseudomonas putida*) and YieF (from *Escherichia coli*) were examined. ChrR transferred >25% of the NADH electrons to ROS and probably generates Cr(V), but only transiently. ChrR *Pseudomonas putida* mutants protects against chromate toxicity by minimizing ROS generation. However, YieF Cr reductase transferred only 25% of the NADH electrons to ROS. Therefore, it was suggested that YieF may be an even more suitable candidate for further studies than ChrR [33].

Although several bacterial Cr resistance mechanisms were reported, the best characterized mechanisms comprise efflux of chromate ions from the cell cytoplasm and reduction of Cr(VI) to Cr(III). For example, efflux of chromate by the ChrA transporter has been established in *Pseudomonas aeruginosa* and *Cupriavidus metallidurans* (formerly *Alcaligenes eutrophus*) and consists of an energy dependent process driven by the membrane potential. However, the CHR protein family, which includes putative ChrA orthologs, currently contains about 135 sequences from all three domains of life can reduce chromate by chromate reductases from diverse bacterial species generating Cr(III) that may be detoxified by other mechanisms [34].

5. Conclusion

Pseudomonas stutzeri (PS) and 3 sub strains of *Bacillus cereus* ATCC 14579 were subjected to 4 elevated Cr levels (2000-5000 mg/l) for 9 days. Results proved the highest affinity of PS (≈ 80%) to bioaccumulate Cr at initial concentration of 5000 mg/l. Moreover, PS exhibited the superior acclimatization ability against Cr even at the highest Cr concentration reflecting its high Cr resistance with 25% growth stimulation at 4000 mg Cr/l and the lowest growth inhibition (37.1% at 2000 mg Cr/l). On the other hand, Cr has remarkable inhibition activity (75 to 99.7%) on the growth of *Bacillus cereus* strains even at the lowest tested Cr concentration without any stimulation at all the tested concentrations. Therefore, PS was considered highly efficient candidate for Cr removal and was selected to be molecularly characterized. Results demonstrated that ChrT gene responsible for chromate reduction ability was most probably present in *Pseudomonas stutzeri* genome.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under Grant No. (D-100-247-1437). The authors, therefore, acknowledge with thanks DSR technical and financial support.

References

[1] Ahalya N, Ramachandra TV and Kanamadi RD. Biosorption of heavy metals. *Research Journal of Chemistry and Environment*, 7: 71-78, 2003.

[2] Gokhale SV, Jyoti KK and Lele SS. Kinetic and equilibrium modeling of chromium (VI) biosorption on fresh and spent *Spirulina platensis* and *Chlorella vulagris* biomass. *Bioresource Technology*, 99: 3600-3608, 2008.

[3] Lakshmaraj L, Gurusamy A, Gobinath MB and Chandramohan R. Studies on the biosorption of hexavalent chromium from aqueous solutions by using boiled mucilaginous seeds of *Ocimum americanum*. *Journal of Hazardous Materials*, 169: 1141-1145, 2009.

[4] Dakiky M, Khamis M, Manassra A and Mereb M. Selective adsorption of chromium (VI) in industrial wastewater using low cost abundantly available adsorbents. *Advanced Environmental Research*, 6: 533-540, 2002.

[5] Perez MAB, Aguilar MI, Meseguer VF, Ortuno JF, Saez J, et al. Biosorption of chromium (III) by orange (*Eitrus cinesis*) waste: batch and continuous studies. *Chemical Engineering Journa*l, 155: 199-206, 2009.

[6] Wang J and Chen C. Biosorption of heavy metal by *Saccharomyces cerevisiae*: a review. *Biotechnology Advances*, 24. 427-451, 2006.

[7] Mishara SH and Doble M. Novel chromium tolerant microorganisms: isolation characterization and their biosorption capacity. *Ecotoxicology and Environmental Safety*, 71. 874-879, 2008.

[8] Gupta VK and Rastogi A. Biosorption of hexavalent chromium by raw and acid treated green alga *Oedogonium hatei* from aqueous solutions. *Journal of Hazardous Materials*, 163. 396-402, 2009.

[9] Donmez G and Aksu Z. Bioaccumulation of copper (II) and nickel (10) by the non-adapted and adapted growing *Candida* sp. *Journal of Water Research*, 35: 1425-1434, 2001.

[10] Srividya K and Mohanty K. Biosorption of hexavalent chromium from aqueous solutions by *Catla catla* scales: Equilibrium and kinetics studies. *Chemical Engineering Journal*, 155: 666-673, 2009.

[11] Seker A, Shahwan T, Eroglu AE, Yilmaz S, Demirel Z, et al. Equilibrium thermodynamic and kinetic studies for the biosorption of aqueous lead (II) Cadmium (II) and nickel (II) ions on *Spirulina platensis*. *Journal of Hazardous Materials*, 154: 973-980, 2008.

[12] Elizabeth KM and Anuradha TVR. Biosorption of hexavalent chromium by non-pathogenic bacterial cell preparations. *Indian Journal of Microbiology*, 40: 263-265, 2000.

[13] Cheung KH and Dong Gu JI. Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: A review. *International Biodeterioration and Biodegradation*, 59: 8-15, 2007.

[14] Batool R, Yrjälä K and Hasnain S. Alleviation of phyto-toxic effects of chromium by inoculation of chromium (VI) reducing *Pseudomonas aeruginosa* Rb-1 and *Ochrobactrum intermedium* Rb-2. *International Journal of Agriculture and Biology*, 17: 21-30, 2015.

[15] Batool R, Yrjäla K and Hasnain S. Hexavalent chromium reduction by bacteria from tannery effluent. *Journal of Microbiology and Biotechnology*, 22: 547-554, 2012.

[16] Pal S and Vimala Y. Bioremediation of chromium from fortified solutions by *Phanerochaete Chrysosporium* (MTCC 787). *Journal of Bioremediation and Biodegradation*, 2: 5, 2011.

[17] Clesceri LS, Greenberg CG and Eaton AD (eds.). Standard Method for the Examination of Water and Wastewater, 20th edn. American Water Work Association, Water Environment Federation, Washington, DC, 1999, APHA-AWWA-WEF. USA, 1999.

[18] Deng P, Tan X, Wui Y, Bai Q, Jia Y and Xiao H. Cloning and sequence analysis demonstrate the chromate reduction ability of a novel chromate reductase gene from *Serratia* sp. *Experimental and Therapeutic Medicine*, 9: 795-800, 2015.

[19] Zuki AA, Mohammed MA, Zain BM Md and Yaakop S. Molecular evidence on symbiotic relationships of *Bracovirus* and *Cotesia* species based on PTP R Region. *Journal of Life Sciences and Technologies*, 3(1): 16-19, 2015.

[20] Sambrook J, Fritsch EF and Maniatis T. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, M.A: Cold Spring Harbor Laboratory Press, 1987.

[21] Arbeli Z and Fuentes C. Prevalence of the gene trzN and biogeographic patterns among atrazine-degrading bacteria isolated from 13 Colombian agricultural soils. *FEMS Microbiology Ecology*, 73: 611-623, 2010.

[22] Elangovan R, Philip L and Chandraraj K. Hexavalent chromium reduction by free and immobilized cell-free extract of *Arthrobacter rhombi*-RE. *Applied Biochemistry and Biotechnology*, 160: 81-97, 2010.

[23] Zhao C, Yang Q, Chen W and Teng B. Removal of hexavalent chromium in tannery wastewater by *Bacillus cereus*. *Canadian Journal of Microbiol*ogy, 58: 23-28, 2012.

[24] Soni S, Singh R, Awasthi A and Kalra A. A Cr(VI)-reducing *Microbacterium* sp. strain SUCR140 enhances growth and yield of *Zea mays* in Cr(VI) amended soil through reduced chromium

toxicity and improves colonization of arbuscular mycorrhizal fungi. *Environmental Science and Pollution Research*, 21(3):, 1971-1979, 2014.

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

[26] He D, Zheng M, Ma T, Li C and Ni J. Interaction of Cr(VI) reduc- tion and denitrification by strain *Pseudomonas aeruginosa* PCN-2 under aerobic conditions. *Bioresour Technol*, 185: 346-352, 2015.

[27] El-Bestawy E, Al-Fassi F, Amer R and Aburokba, R.. Biological Treatment of leather-tanning industrial wastewater using free living bacteria. *Advances in Life Science and Technology*, 12: 46-65, 2013.

[28] El-Bestawy E and Aburokba R. Efficiency of *Pseudomonas stutzeri* strain M15-10-3 in the treatment of leather tanning industrial wastewater using gravel biofilm system. *Desalination & Water Treatment*, 91: 175-184.

[29] Thatoi H, Das S, Mishra J, Rath BP and Das N. Bacteria chromate reductase, a potential enzyme for bioremediation of hexavalent chromium: A review. *Journal of Environmental Management*, 146: 383-399, 2014.

[30] Liu X, Wu G, Zhang Y, Wu D, Li X and Liu P. Chromate reductase YieF from *Escherichia coli* enhances hexavalent chromium resistance of human HepG2 Cells. *International Journal of Molecular Sciences*, 16: 11892-11902, 2015.

[31] Tan X, Deng P, Wu Y, Bai Q, Jia Y and Xiao H. In vitro synthesis and activity identification of Chromate reductase T. *Chongqing Yike Daxue Xuebao* 39: 973-977, 2014 (In Chinese)

[32] Zhou S, Dong L, Deng P, Jia Y, Bai Q, Gao J and Xiao H. Reducing capacity and enzyme activity of chromate reductase in a ChrT-engineered strain *Experimental and Therapeutic Medicine*, 14: 2361-2366, 2017.

[33] Ackerley DF, C. F. Gonzalez CF, Park CH, Blake R, Keyhan M and Matin A. Chromate-Reducing Properties of Soluble Flavoproteins from *Pseudomonas putida* and *Escherichia coli*. *Applied and Environmental Microbiology*, 70(2): 873-882, 2004.

[34] Martha I. Ramırez-Dıaz MI, Dıaz-Perez C, Vargas E, Riveros-Rosas H, Campos-Garcıa J and Cervantes C. Mechanisms of bacterial resistance to chromium compounds. *Biometals*, 21: 321-332, 2008.