



DOI: <https://doi.org/10.54692/lgujls.2022.0603221>

Research Article

LGU J. Life. Sci

Vol 6 Issue 3 July - September 2022

ISSN 2519-9404

eISSN 2521-0130

Reduction of Chromium (VI) by indigenous bacteria isolated from industrial effluents of Pakistan

Sabeen Sabri^{*1}, Rida Batool², Muhammad Shoaib Akhtar¹

1. Department of Microbiology and Molecular Genetics, Faculty of Life Sciences, University of Okara, Okara 56130 Pakistan
2. Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore 54590 Pakistan

Corresponding Author's Email: sabin.sabri@uo.edu.pk

ABSTRACT: *The present study was aimed to isolate and examine the reduction potential of chromium resistant bacteria. For this purpose, 10 chromium resistant bacterial strains were isolated from three different samples, collected from effluents of industries. These isolated strains were designated as A, B₁, B₂, C, L₁, L₂, L₃, M, I₁, and I₂. All strains were able to resist up to 3500 µg/ml of potassium chromate on L-agar. At optimum pH 7 and temperature 37 °C, all isolated strains showed optimum reduction potential. The strain B₁ and L₃ showed 97% reduction potential in DE broth while B₁ in L-broth and I₂ in acetate minimal broth showed 98% and 97% reduction potential respectively. In the case of artificial sewage water, strain M and L₂ showed 94% reduction potential. In sample-1 of domestic sewage water (sterile), B₂ showed 94% while I₂ (non-sterile) had maximum reduction potential. Sample-2 (A, C, L₂, I₁) from domestic sewage water (non-sterile) had 96% and A, C, L₂, I₁ (sterile) also showed a 96% maximum reduction potential.*

Keywords: Sewage Water, Reduction Potential, Chromium VI, Heavy Metal, Bacteria.

INTRODUCTION

In developing regions of the world, a variety of industrial effluents containing toxic metals are released into the environment without degradation, (Dixit

et al., 2015) which is the major cause of rapidly increasing environmental pollution (Badmus et al., 2007). Major heavy metals like chromium as well as its compounds are extensively used in

various industries. Cr is used in the processing as well as finishing of leather. It is also employed in alloy manufacturing, production of nuclear weapons and chromic acid, cleaning of pigments before electroplating, drilling mud, catalytic manufacture, refractory steel production, and textile dyeing (Priester et al., 2006; Ghani, 2011). Cr is found to exist in various oxidation states, but both the hexavalent (VI) as well as trivalent (III) species of Cr, are found to be most stable in the environment (Opperman and van Heerden, 2008; Helena Oliveira, 2012). The oxidation state of chromium greatly influences its biological effects (Opperman and van Heerden, 2007).

Conventional methods used to detoxify or eliminate the hazardous CrO_4^{2-} are ion exchange, evaporation recovery, solvent extraction, kaolinite, adsorption, chemical reduction and reverse osmosis. These methods have many disadvantages including incomplete metal removal, high cost, and generation of waste such as toxic sludge which must be removed. Therefore, it is necessary to develop an efficient, cost competitive and eco-friendly method to detoxify heavy metals such as chromium to protect human health and the environment (Saxena et al., 2016). The use of microorganisms is an

efficient alternative to traditional techniques for the degradation of hazardous heavy metals (Igiri et al., 2018). Various Cr-resistance mechanisms exhibited by the microbes include precipitation, biosorption, chromate efflux, diminished accumulation, and reduction of hexavalent Cr to trivalent Cr (Opperman and van Heerden, 2007; Ayele and Godeto, 2021). Chemical, as well as biological mechanisms are used for chromium (VI) reduction (Kourtev et al., 2006). The toxic Cr (VI) is reduced to a less toxic form of Cr (III) in the reduction process (Bandara et al., 2020). Microorganisms can reduce the Cr (VI) directly and indirectly through microbial metabolism and bacterial metabolites respectively (Ahemad, 2015; Samuel et al., 2013). Samples were collected from the waste of industries located along G.T. Road (Lahore), Band Road (Lahore), and a tannery (in Kasur), Pakistan on the media supplemented with chromium. Chromium reducing bacteria were isolated from evaporation ponds, industrial sewage, discharged water, and tannery sludge. M and B₁ strains were found to be from the genus *Azotobacter*. Strain A and B showed characteristics similar to the genus *Bacillus* while C, I₁, I₂, L₁, L₂, and L₃ strains showed

attributes like the genus *Pseudomonas*. Currently, this study was aimed to isolate and examine the reduction potential of chromium-resistant bacteria. Because bacteria have a better ability to reduce the highly toxic form of Cr (VI) than other microorganisms. Our further objective is to analyze the capacity of hexavalent Cr reduction displayed by the isolated native hexavalent chromium resistant strains of bacteria.

MATERIALS AND METHODS

Isolation of Cr Resistant Bacterial Strains

Different samples (S₁, S₂, S₃) were inoculated in L-agar plates containing 500, 1000, and 1500 µg/ml of potassium chromate. All these plates were incubated at 37°C for 1-2 days. The plates were examined for the growth of Cr resistant strains after 24 hours. From each sample, various Cr-resistant colonies of bacterial strains were selected based on morphology.

Biochemical and Morphological Characterization of the Cr (VI) Resistant Bacterial Strains

Different colonies were separated by using the direct plate method. The morphological characteristics of different colonies were examined under a microscope. Cell, as well as colony morphology, was also performed. Various biochemical tests such as

Voges, Proskauer, Nitrate Reduction, Methyl Red, Starch Hydrolysis, Oxidation Fermentation, catalase, oxidase, and Urease were performed to characterize the Cr-resistant strains of bacteria (Murray, 1994).

Reduction Potential Estimation

The Cr (VI) reduction potential was observed in Deleo and Ehrlich chromium reduction media (DeLeo and Ehrlich, 1994), acetate minimal broth, and L-broth (Pattanapitpaisal et al., 2001; Mahzer et al., 2020;) . L-broth with pH 7.0 was prepared and mixed with different chromium salts i.e., 100, 700, and 1500 µg/ml. Except for the control, each test tube was inoculated with a day-old bacterial culture. After inoculation, all test tubes were incubated for 1 day at 37°C and 150rpm. Tubes were observed for reduction potential after 24 hours. In domestic as well as artificial sewage water (Peitzsch et al., 1998) Cr-reduction was observed. 2 samples of domestic sewage water taken from 2 different sources were used. The pH of sample 1 and sample 2 was 6-7 and 5-7 respectively. Sewage water (non-sterile) was used in 1st experiment but sterile sewage water was used in 2nd experiment for inoculation. After inoculation, the sewage water was incubated at 150 rpm for 7 days. The

Diphenyl Carbazide method was used for estimating the reduction potential.

Effect of Environmental Factors on the Reduction Potential

The effect of temperature variation (28 °C, 37°C & 42°C) and pH ((5, 7, 9, and 11) on the reduction potential of Cr-resistant bacteria were examined. Respective Cr reducing strains of bacteria were inoculated in L-broth containing 100, 700, and 1500 µg/ml of potassium chromate. To examine the pH effect, the test tubes were incubated for 24 hours at 37°C, and for temperature, the test tubes were incubated at the corresponding temperature. The

reduction potential was checked after incubation by the Diphenyl carbazide method.

RESULTS

Isolated Chromium (VI) Resistant Bacterial Strains

Three different samples of wastewater were collected from the waste of industries located along G.T. Road (sample 1), Band Road (sample 2), and tannery (sample 3) in Kasur (Table 1). 10 Cr-resistant strains of bacteria were isolated from these samples. The strains were named A, B₁, B₂, C, I₁, I₂, L₁, L₂, L₃, and M.

Table 1: Bacteria Strains Isolated from waste of different Industries in Pakistan

Sample	Location/source	Material	Strain isolated
S₁	Ittehad chemicals, G.T road Lahore	Wastewater	A, B ₁ , B ₂ , and C
S₂	Band Road, Lahore	Wastewater	L ₁ , L ₂ , L ₃ , and M
S₃	Tannery effluents, Kasur	Wastewater	I ₁ and I ₂

A, B₁, B₂, and C strains of bacteria were isolated from sample 1 (S₁). L₁, L₂, L₃, and M strains were isolated from sample 2 (S₂) while sample 3 (S₃), I₁, and I₂ strains of bacteria were isolated.

Biochemical and Morphological Characteristics of the Cr (VI) Resistant Strains

The texture, shape, color, light transparency, size (mm), elevation, and margin of all colonies were examined (Table 2).

Table 2: Colony morphology of Cr reducing bacteria isolated from different samples of wastewater

Sample	Bacterial strains	Elevation	Gram reaction	Size (mm)	Shape	Motility	Texture	Margin	Color	Light transparency	Cell arrangement	Cell morphology
S1	A	Raised	+VE	2.0	Round	+	Shiny	Entire	Cream	TL	Chains	Rods
	B ₁	Flat	-VE	4.0	Irregular	+	Shiny	Entire	C.Y.	TL	Clumps	Cocci
	B ₂	Convex	+VE	5.0	Round	+	Mucoid	Entire	T.S.	TL	Scattered	Rods
	C	Convex	-VE	3.0	Round	+	Mucoid	Entire	O.W.	TL	Scattered	Rods
S2	L ₁	Flat	-VE	1.0	Round	+	Shiny	Wavy	Cream	TL	Scattered	Rods
	L ₂	Flat	-VE	2.0	Irregular	+	Shiny	Wavy	Cream	TL	Diplobacilli	Rods
	L ₃	Raised	-VE	1.0	Round	+	Dry	Entire	O.W.	TL	Chains	Rods
	M	Raised	-VE	4.0	Irregular	+	Shiny	Entire	Yellow	TL	Clumps	Cocci
S3	I ₁	Flat	-VE	0.1	Round	+	Dry	Entire	O.W.	TL	Chains	Rods
	I ₂	Flat	-VE	0.1	Round	+	Dry	Entire	Cream	TL	Scattered	Rods

C.Y., Creamy Yellow; T.S., Transparent Shiny; O.W., Off White; TL, Translucent; + VE, Positive; - VE, Negative

Different biological, as well as chemical activities, were exhibited by bacterial strains and shown in Table 3.

Table 3: Biochemical features of Cr Reducing Bacteria

Sample	Bacterial strains	Urease	Oxidase	Nitrate reduction	Catalase	Starch hydrolysis	MR	VP	OF	
									A	G
Sample 1	A	+	+	+	+	-	-	-	-	-
	B ₁	-	-	+	+	-	-	+	+	-
	B ₂	+	+	+	+	-	-	+	-	-
	C	+	+	+	+	-	-	-	-	-
Sample 2	L ₁	+	+	-	+	-	-	-	-	-
	L ₂	+	+	-	+	-	-	-	-	-
	L ₃	-	+	+	+	-	+	+	-	-
	M	-	+	+	+	-	+	-	+	-
Sample 3	I ₁	-	+	+	+	-	+	-	-	+
	I ₂	-	+	+	+	-	-	-	-	-

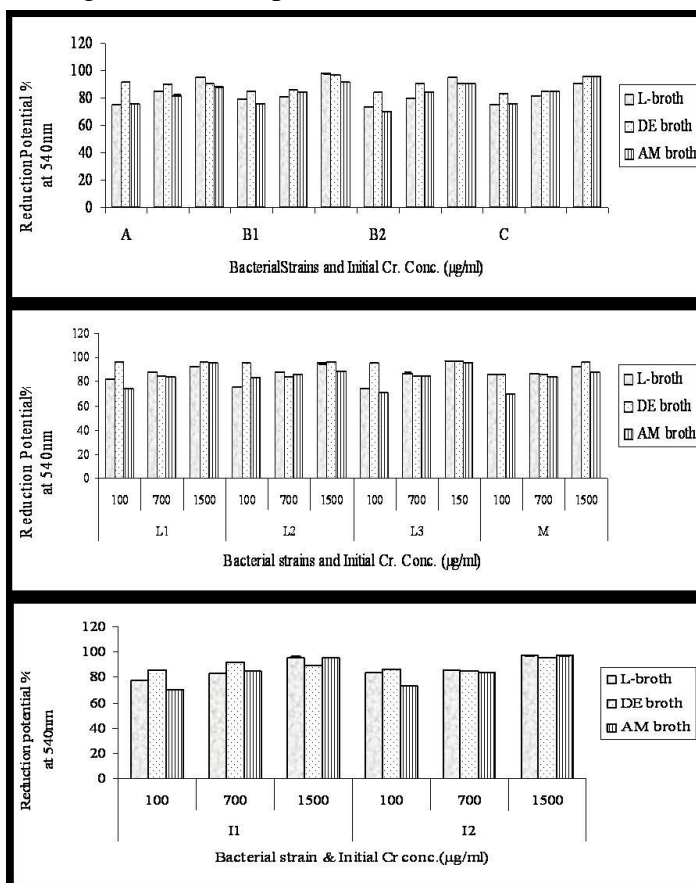
A: Acid Production; VP: Voges Proskauer; -: Negative; +: Positive; MR: Methyl Red; G: Gas Production

Estimation of Cr (VI) Reduction Potential

Reduction Potential in Different Medias

The isolated Cr-resistant strains of bacteria were capable of reducing the toxic hexavalent Cr into trivalent Cr which is less toxic. Maximum reduction potential was observed by all strains in DeLeo and Ehrlich media while among the samples of sewage water, sample-1

showed the highest reduction potential (domestic sewage water). It was also noted that reduction potential increases by increasing the Cr (VI) initial concentration. In DeLeo and Ehrlich media, the isolated strains showed ~ 83% - ~97% reduction potential while ~ 45% - ~98% and ~70% - ~97% in L-broth and acetate minimal broth respectively (Fig. 1).



L: Luria Broth; DE: DeLeo and Ehrlich Media; AM: Artificial Media

Fig. 1. Reduction potential estimation of Cr reducing bacterial strains that are isolated from three different samples in various media

Estimation of Reduction Potential in Artificial and Domestic Sewage water

In the sample of domestic non-sterile sewage water, all inoculated strains exhibited the highest reduction potential. In the case of sample-1 from non-sterile household sewage water, ~ 91% - ~96% while in sample-1 from domestic sterile sewage water, ~ 68% -

~98% reduction potential was recorded. However, in sample-2 (non-sterile household sewage water) ~66% - ~96%, and in sample-2 (sterile domestic sewage water) the reduction potential was ~35% - ~96%. While in the case of a sample of artificial sewage water the reduction potential was ~36% - 94% (Fig. 2).

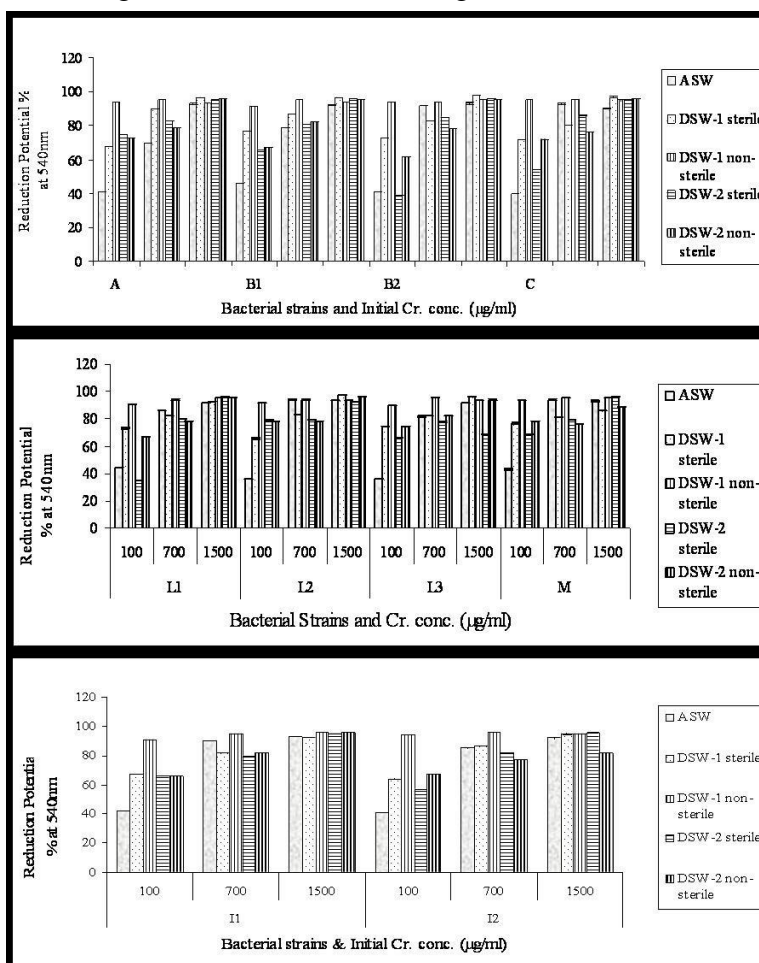


Fig. 2. Reduction potential estimation of Cr reducing bacterial strains that are isolated from three different samples in artificial as well domestic sewage water

ASW: Artificial Sewage Water; DSW: Domestic Sewage Water

Effect of pH and Temperature on Reduction Potential

Environmental factors also affect the growth of bacteria, so the reductional potential was recorded by varying the temperature and pH.

Reduction potential was ~59% - ~98% at pH 5 while at pH 7.0 the reduction potential was ~ 86% - ~97%. However, at pH 9 and 11 the reduction potential was ~ 52% - ~98% and ~50% - ~98% respectively (Fig. 3).

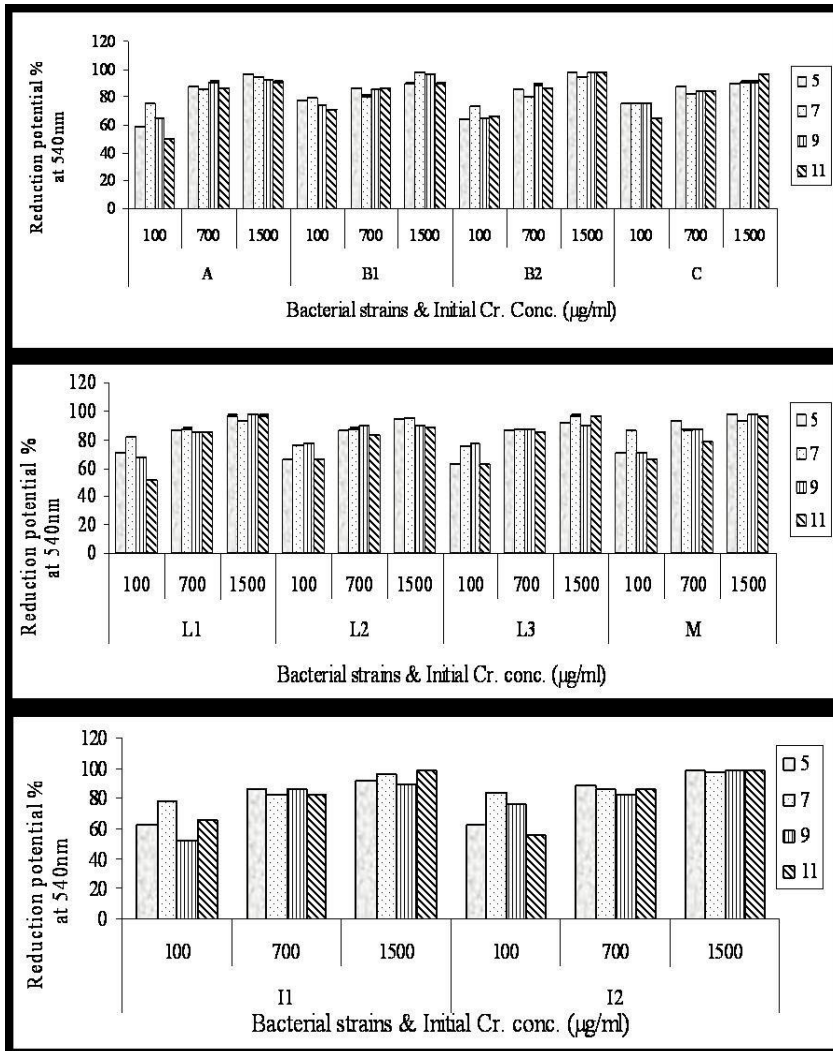


Fig. 3. Effect of variation in pH on the reduction potential of Cr reducing strains that are isolated from three different samples

The results revealed that the capability of Cr-resistant strains to change the toxic form of Cr (VI) into Cr (III) which

is less toxic, was greatly influenced by temperature. The reduction potential varied as variation occurred in

temperature. The reduction potential was ~60% - ~98%, ~75% - ~98% and ~43% ~-98% at 42°C, 37 °C and 28 °C respectively (Fig. 4).

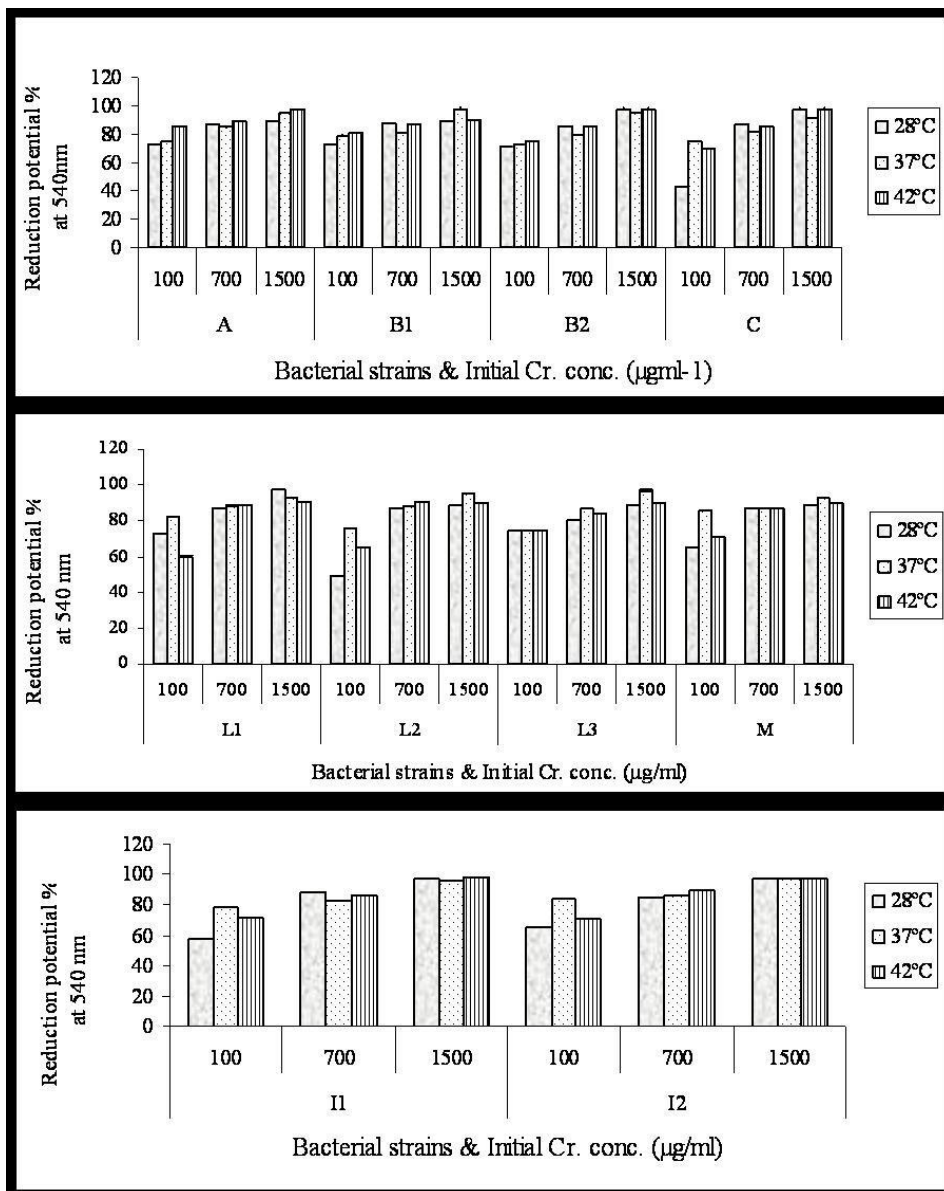


Fig. 4. Effect of variation in temperature on the reduction potential of Cr reducing strains that are isolated from three different samples

DISCUSSION

An increasing number of industries is one of the major reasons for air as well as water pollution (Beauchemin et al.,

2007; Heede, 2006; Oliveira, 2012; Sanchez-Segado et al., 2015) and chemicals used by the industries are released into the environment (Proshad

et al., 2018). As chromium is extremely used in many industries especially in electroplating so it is a usual contaminant of groundwater as well as surface water. Chromium can be reduced either naturally from the solution by using different reductants like aqueous Fe (II) or by *in situ* reduction (Ellis et al., 2002).

In the present work, hexavalent Cr-resistant strains of bacteria were isolated from effluents of different industries in Pakistan. Isolation of these bacteria is also reported in various studies (Mustapha and Halimoon, 2015; Marzan et al., 2017). 10 Cr (VI) resistant strains (A, B₁, B₂, C, L₁, L₂, L₃, M, I₁, and I₂) were isolated by using L-agar plates containing 500, 1000, and 1500 µg/ml of potassium chromate. These isolated strains of bacteria were capable of tolerating more than 3500 µg/ml of potassium chromate in media. Different workers isolated these Cr (VI) strains. Chromium resistant strains reported by the workers were able to tolerate 40 mg/ml (Opperman and van Heerden, 2007) and 150 µg/ml (Ellis et al., 2002). Different attributes based on the morphology were obtained among the isolated strains. Also, for the characteristics of morphological diversity, 10 viable strains of bacteria were compared.

Differences, as well as variations, were noted between the isolated strains, which belong to 3 different samples of sewage water and few variations for various tests were observed in the biochemical properties of isolated strains. Bacteria can reduce the toxic Chromium (VI) into less toxic Chromium (III), but still need to improve their activity at the cellular and enzymatic levels (Ackerley et al., 2004; Justin Samuel et al., 2013; Ahemad, 2015). With the increase of chromium concentration, the reduction potential also increased. It was recorded that in DeLeo and Ehrlich media, the reduction potential exhibited by the isolated strains of bacteria was ~ 83% - ~97% while in Acetate minimal and L-broth, the reduction potential was ~70% - ~97% and ~ 45% - ~98% respectively.

CONCLUSION

It was concluded that isolated indigenous Cr reducing strains of bacteria are not only capable of resisting the Cr (VI) but also has potential to reduce the toxic Cr (VI) under variable conditions of bacterial growth. These strains of bacteria can be employed for the bioremediation of the environment, contaminated by chromium.

Acknowledgment

The authors would like to acknowledge the University of the Punjab, Lahore,

Pakistan for providing financial support for this study.

REFERENCES

1. Ackerley DF, Gonzalez CF, Park CH, Blake R, Keyhan M, Matin A (2004). Chromate-reducing properties of soluble flavoproteins from *Pseudomonas putida* and *Escherichia coli*. *Appl. Environ. Microbiol.* 70: 873-882.
2. Ahemad M (2015). Enhancing phytoremediation of chromium-stressed soils through plant-growth-promoting bacteria. *J. Gen. Eng. Biotechnol.* 13: 51-58.
3. Ayele A, Godeto YG (2021). Bioremediation of chromium by microorganisms and its mechanisms related to functional groups. *J. Chem.* 2021: 7694157.
4. Badmus M, Audu T, Anyata B (2007). Removal of heavy metal from industrial wastewater using hydrogen peroxide. *Afr. J. Biotechnol.* 6: 238-242.
5. Bandara PC, Pena-Bahamonde J, Rodrigues DF (2020). Redox mechanisms of conversion of Cr(VI) to Cr(III) by graphene oxide-polymer composite. *Sci. Rep.* 10: 9237.
6. Beauchemin K, McGinn S, Martinez T, McAllister T (2007). Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. *J. Anim. Sci.* 85: 1990-1996.
7. DeLeo PC, Ehrlich HL (1994). Reduction of hexavalent chromium by *Pseudomonas fluorescens* LB300 in batch and continuous cultures. *Appl. Microbiol. Biotechnol.* 40: 756-759.
8. Dixit S, Yadav A, Dwivedi PD, Das M (2015). Toxic hazards of leather industry and technologies to combat threat: a review. *J. Clean. Produc.* 87: 39-49.
9. Ellis AS, Johnson TM, Bullen TD (2002). Chromium isotopes and the fate of hexavalent chromium in the environment. *Sci.* 295: 2060-2062.
10. Ghani AGA (2011). Effect of chromium toxicity on growth, chlorophyll and some mineral nutrients of brassica juncea L. *Egypt. Acad. J. Biol. Sci.* 2: 9-15.
11. Heede R (2006). LNG supply chain greenhouse gas emissions for the Cabrillo deepwater port: natural gas from Australia to California. Climate Mitigation Services. Available online: http://www.edcnet.org/pdf/Heede_06_LNG_GHG_Anlys.pdf (accessed on 3 November 2011).

12. Igiri BE, Okoduwa SIR, Idoko GO, Akabuogu EP, Adeyi AO, Ejiogu IK (2018). Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater: Rev. J. Toxicol. 2568038.
13. Kourtev PS, Nakatsu CH, Konopka A (2006). Responses of the anaerobic bacterial community to addition of organic C in chromium (VI)-and iron (III)-amended microcosms. Appl. Environ. Microbiol. 72: 628-637.
14. Mahzer M, Gohar M, Mazher S (2020). Plasmid Profiling and Effect of Different Physiological Parameters on the Chromium Reduction Potential of Microbes. J. Microbiol. Mole. Gene. 1: 19-31.
15. Marzan LW, Hossain M, Mina SA, Akter Y, Chowdhury AM (2017). Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: Bioremediation viewpoint. Eglyp. J. Aquat. Res. 43(1): 65-74.
16. Murray R (1994). Determinative and cytological light microscopy. Meth. Gen. Mol. Bacteriol. 7-20.
17. Mustapha MU, Halimoon N (2015). Screening and isolation of heavy metal tolerant bacteria in industrial effluent. Proce. Environ. Sci. 30: 33-7.
18. Oliveira H (2012). Chromium as an environmental pollutant: insights on induced plant toxicity. J. Bot. 375843.
19. Opperman DJ, van Heerden E (2008). A membrane-associated protein with Cr(VI)-reducing activity from *Thermus scotoductus* SA-01. FEMS Microbiol Lett, 280: 210-218.
20. Opperman, DJ, van Heerden E (2007). Aerobic Cr(VI) reduction by *Thermus scotoductus* strain SA-01. J. Appl. Microbiol. 103: 1907-1913.
21. Pattanapitpaisal P, Brown NL, Macaskie LE (2001). Chromate reduction and 16S rRNA identification of bacteria isolated from a Cr(VI)-contaminated site. Appl. Microbiol. Biotechnol. 57: 257-261.
22. Peitzsch N, Eberz G, Nies DH (1998). *Alcaligenes eutrophus* as a bacterial chromate sensor. Appl. Environ. Microbiol. 64: 453-458.
23. Priester JH, Olson SG, Webb SM, Neu MP, Hersman LE, Holden PA (2006). Enhanced exopolymer production and chromium stabilization in *Pseudomonas putida*

- unsaturated biofilms. *Appl. Environ. Microbiol.* 72: 1988-1996.
24. Proshad R, Kormoker T, Mursheed N, Islam MM, Bhuyan MI, Islam MS, Mithu TN (2018). Heavy metal toxicity in agricultural soil due to rapid industrialization in Bangladesh: a review. *Int. J. Adv. Geosci.* 6: 83-88.
25. Samuel J, Paul ML, Ravishankar H, Mathur A, Saha DP, Natarajan C, Mukherjee A (2013). The differential stress response of adapted chromite mine isolates *Bacillus subtilis* and *Escherichia coli* and its impact on bioremediation potential. *Biodegrad.* 24: 829-842.
26. Samuel J, Paul ML, Ravishankar H, Mathur A, Saha DP, Natarajan C, Mukherjee A (2013). The differential stress response of adapted chromite mine isolates *Bacillus subtilis* and *Escherichia coli* and its impact on bioremediation potential. *Biodegrad.* 24: 829-842.
27. Sanchez-Segado S, Makanyire T, Escudero-Castejon L, Hara Y, Jha A (2015). Reclamation of reactive metal oxides from complex minerals using alkali roasting and leaching—an improved approach to process engineering. *Green Chem.* 17: 2059-2080.
28. Saxena G, Chandra R, Bharagava RN (2016). Environmental pollution, toxicity profile and treatment approaches for tannery wastewater and its chemical pollutants. *Rev. Environ. Contam. Toxicol.* 24031-69.