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Amelioration of Cr (VI) into Cr (III) by Some Heavy Metal Resistant Bacterial Strains Isolated from Naran Valley

Ifrah Fayyaz
Institute of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan

Asma Kalsoom
Institute of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan, asmakalsoom824@gmail.com

Rida Batool
Institute of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan, rida.mmg@pu.edu.pk

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AMELIORATION OF Cr (VI) INTO Cr (III) BY SOME HEAVY METAL RESISTANT BACTERIAL STRAINS ISOLATED FROM NARAN VALLEY

IFRAH FAYYAZ, ASMA KALSOOM AND RIDA BATOOL*

Institute of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan

Corresponding author's email: rida.mmg@pu.edu.pk

ABSTRACT

Total thirty-two strains were isolated from both water and soil samples collected from Naran Valley which is surrounded by the rugged mountains, located in the province of Khyber Pakhtunkhwa, Pakistan. Streak plate method was used to screen the bacterial strains for resistance against selected heavy metals like chromium (Cr), cobalt (Co), copper (Cu), nickel (Ni) and zinc (Zn). Resistance against multiple heavy metals was shown by all the strains, but S15 and W15 were significant as they exhibited a maximum resistance to chromium and copper (1200 µg/ml). These strains also presented multiple antibiotic (ampicillin, tetracycline, chloramphenicol, and erythromycin) resistance patterns. Bacterial strain W15 exhibited 90 % whereas, S15 exhibited 80 % chromium removal at an initial concentration of 800 µg/ml of chromate within 48 h of incubation. Inductive enzyme activity was shown by both the strains. 16S rRNA gene sequencing of strains S15 and W15 revealed the homology with *Microbacterium* sp. and *Pseudomonas* sp., respectively. These two multiple metal resistant strains can be further exploited for the remediation of metal polluted sites.

Keywords: Heavy metals, Cr (VI) pollution, indigenous bacteria, naran valley.

INTRODUCTION

Soil act as a medium where certain harmful elements recognized as heavy metals such as chromium, nickel, lead and cadmium become submerged and remain there for so long that they affect the flora and fauna of soil (Nicholson et al., 2003). The anthropogenic activities, creating water pollution are sewage and industrial wastewater, organic matter of animals and plants, domestic waste, surface washing, agricultural and chemical wastes (Lokeshwari and Chandrappa 2006). Transition metal accumulation in plants and soil is the main concern nowadays due to its potential toxicity to the food chain. When heavy metal pollution crosses the threshold limit, it intensifies the levels of the contaminants in the environment causing a threat to the food chain (Ivezić et al., 2013). Poor animal husbandry techniques, irrigating fields with poor quality water, overgrazed grasslands, application of fertilizers and excessive use of pesticides, are all examples of agricultural operations that can pollute the environment (Khatri and Tyagi 2015). These actions have resulted in the accumulation of harmful metals in soil which enters into the food chain through plants via root channels and ultimately affects human health (Simeonov et al., 2003). The top portion (25 cm) in soil mostly composed of toxic metals surrounded by the plant's roots (Freitas et al., 2004). The expansion in industrial, economic, urban growth and inadequate environmental measures has caused the unavailability of irrigation water. To manage this problem in the irrigation sector, domestic and industrial effluents have been previously used as a resource (Chary et al., 2008). The continual process of irrigating crops with wastewater is not only causing the accumulation of metal

ions in the soil but also polluting the plants (Boularbah et al., 2006). When the soil is saturated with heavy metals, they are released into the groundwater and eventually taken up by plants. This process affects the quality and safety of food products (Zhao et al., 2010). In Pakistan, growers practice the utilization of effluent as a substitute for irrigating fields due to freshwater unavailability (Sadiq et al., 2005). Other than Pakistan, countries such as Oman, Morocco, Saudi Arabia, the Middle East and Jorden also follow the act of using wastewater for irrigation purposes (Sato et al., 2013). Food chain contamination is one of the key routes that contribute to 90% of metals ions, compared with other sources, such as contact with the skin and inhalation (Loutfy et al., 2006). Ingestion of foods that are contaminated increases the risk of developing cancer (Arora et al., 2008).

Chromium (Cr) is a naturally occurring element in soil (10-50 mg kg^{-1}). Cr (VI) and Cr (III) are stable oxidation states naturally present in the environment. Cr is widely used in paint and pigments, metal plating, production of steel, leather industry, and wood preservation (Batool et al., 2012). The effluents of these industries carry high concentrations of Cr compounds causing toxicity to living organisms although it is also considered a necessary micronutrient for nucleic acid stabilization, metabolism of glucose, and enzyme system stimulations. Conventional techniques like precipitation, ion exchange, adsorption were typically employed to remove Cr (VI). However, these methods are expensive for energy consumption as well as treatment and disposal cost. Bioremediation involves the elimination of harmful pollutants by microorganisms. Microbes can be particularly helpful in the remediation of chromate contaminated areas since they can convert Cr (VI) into Cr (III). Thus, the presence of heavy metals must be monitored to ensure the safety of human health as well as the environment.

The current study was focused on the isolation of heavy metal resistant bacteria from Naran Valley located in the province of Khyber Pakhtunkhwa, Pakistan. This valley is approximately 270 km away from Islamabad (capital) at 34° 54.26´N to 35° 08.76´ N latitude and 73° 38.90´ E to 74° 01.30` E longitude; elevation between 2450 to 4100 m above mean sea level. This valley is formed by the Rocky Mountains on both sides of Kunhar River, flowing from the north-east to south-west direction down the valley to the Naran city. Physiographic of this area shows that it is situated on the far western periphery of the Himalayan range (Figure 1) (Khan et al., 2012). Naran valley is a famous tourist attraction in the northern region of Pakistan. Tourists from all over the country and around the world visit this valley mainly in the summer every year. This results in the pollution of this beautiful valley as trash is thrown by tourists in the nearby areas. This study investigated the soil and water samples of Naran valley for heavy metal resistant bacterial strains and further purification and analysis of inductive behavior of selected bacteria for chromate removal.

MATERIALS AND METHODS

Isolation of Bacteria

Naran Valley was targeted for the collection of a soil and water sample. The water sample was collected in a sterile bottle from the surface of the Lake Saif ul Malook, a famous tourist spot in the Naran valley and the top layer of soil was collected in the sterile plastic bag from nearby of the lake. The temperature and pH of both samples were recorded at the time of collection. Both the soil and water samples were diluted serially and spread on LB-agar plate. The plates were then placed in an incubator at 37 °C for 24 h.

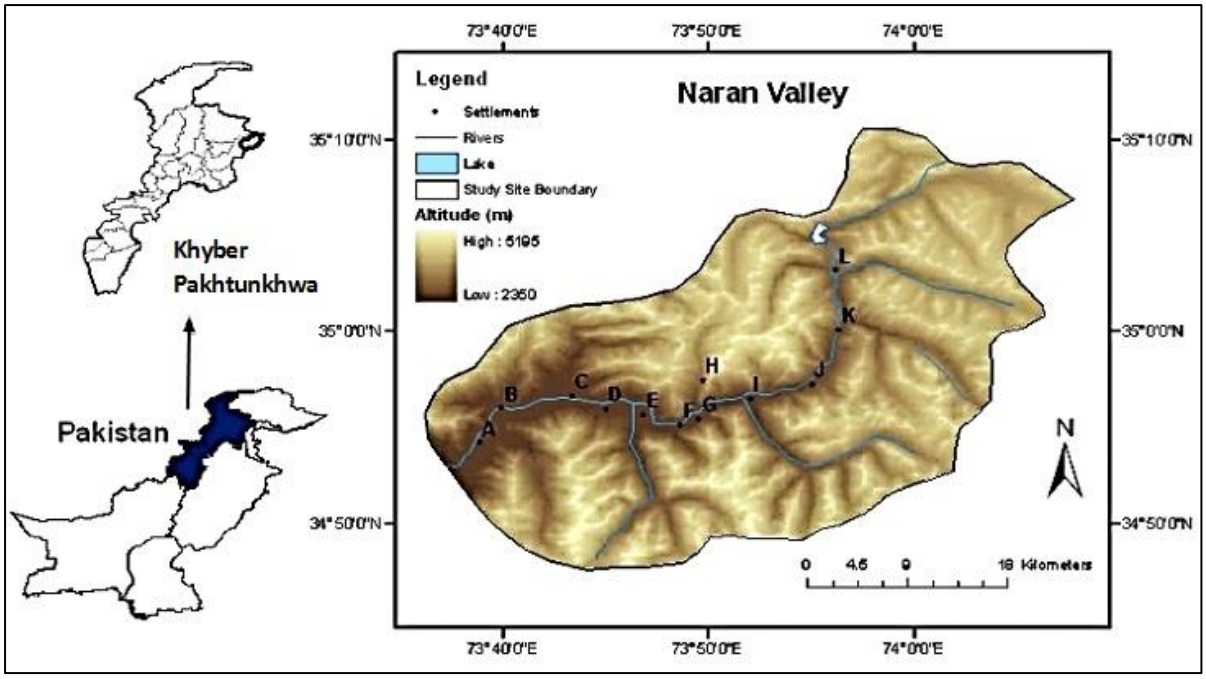

Figure 1: Physiographic map of the Naran Valley; elevation zones, describing its major settlements (A-L), the Kunhar River, originating lake, and the branch watercourses (Khan et al., 2012).

The morphologically different colonies were chosen for further study. Quadrant streaking was done on LB-agar plate to attain pure culture (Zahoor and Rehman 2009).

Identification and Characterization of the Selected Bacteria

For morphological analysis of the thirty-two bacterial strains isolated from water and soil samples, the shape, color,

size, and texture were examined. Gram and spore staining, as well as motility test was also performed. Catalase, oxidase, MR-VP, OF glucose, and mannitol fermentation were done for biochemical characterization according to Bergey's Manual of Determinative Bacteriology (Boone and Castenholz 2001).

Antibiotic Resistance Profiling

Antibiotic resistance of the bacterial isolates was determined by growing the strains on the media supplemented with various antibiotics such as erythromycin, tetracycline, chloramphenicol and ampicillin. Bacterial strains were streaked on LB-agar plates supplied with antibiotics (20-70 µg/ml) to check the resistance against particular antibiotics and the plates were kept in incubator at 37 °C for 24-48 h (Jenkins and Schuetz 2012).

Determination of Minimum Inhibitory Concentration (MIC) of Metal Resistant Bacteria

MIC is the minimum concentration of a compound inhibiting the bacterial growth. To determine the MIC of selected heavy metals, the isolated bacterial strains were streaked on LB-agar medium supplemented with various heavy metals separately (Cr, Co, Cu, Ni and Zn). The plates were kept in an incubator at 37 °C for 24-48 h. The concentration of each heavy metal used was in the range of 200-1400 µg/ml.

Chromate Removal Potential of Selected Bacteria

Selected bacterial strains were evaluated for chromate removal potential by the diphenyl-carbazide method (Batool et al., 2012). Overnight bacterial cultures were centrifuged at 14,000 rpm for 5 min. Distilled water was added in the supernatant, following a few drops of ortho-phosphoric acid and incubated for 15 min at room temperature. Then, optical density was measured at 540 nm. The chromate removal potential was determined by applying the formula below:

% Chromate removal = Ai–Af/Ai × 100

Where, Ai = initial concentration,

Af = final concentration

Effect of Cr (VI) Stress on Inductive Behavior of Selected Bacteria

To determine the effect of chromate stress on the inductive/constitutive behavior of selected bacterial strains, bacterial growth was examined in the LB-broth medium. Two sets of LB-broth were prepared in a 100 ml flask, one supplemented with 1200 µg/ml Cr (VI), and the other was without stress. The flasks were inoculated with 24 h old cultures and incubated for 48 h at 37 °C. One ml of inoculated culture was aseptically drawn from each flask and the absorbance was recorded at 600 nm after the time interval of 6, 12, 18, 24, and 30 h so on till 48 h on a spectrophotometer (Zahoor and Rehman 2009).

16S rRNA Gene Sequencing

Genomic DNA from the selected Cr (VI) resistant bacteria were extracted and sent to Macrogen Inc., Seoul, Korea. 16S rRNA gene sequences of bacterial strains were analyzed and submitted in the National Centre of Biotechnology Information (NCBI) database. For phylogenetic analysis MEGA-X was used and tree was constructed by the neighbor joining method considering the two main domains archaea and eubacteria for bootstrap analysis (Katoh and Standley 2013).

Statistical Analysis

All research work was carried out in triplicates. Data was analyzed statistically as mean ± standard error in Graph PadPrism version 8 and OriginPro 8.5 software.

RESULTS AND DISCUSSION

Isolation and Characterization of the Bacterial Strains

Bacterial strains from a soil and water sample of Naran valley were isolated and screened for multiple heavy metal resistance. The soil sample was dark brown with a muddy appearance and pH 7.0. The water sample showed a pH value of 6.0 and the temperature recorded at the time of sampling was 20 ºC. Organic and inorganic components of the soil are bound to the heavy metals compounds. All the metal contaminated soil and water bodies demonstrated the existence of metal resistant microorganisms. These microbial strains can tolerate higher concentrations of the specific metals which are present in its surrounding and are capable of reducing that particular metal indigenously (Abou-Shanab et al., 2008). A total of thirty-two bacterial strains were isolated from a water and soil sample of Naran valley. Initially, the isolates were differentiated based on Gram's reaction as Gram-positive or Gram-negative species. Out of thirty-two, 21 were gram-positive whereas, 11 were gram-negative bacterial strains. The bacterial strains that showed maximum resistance towards chromium were selected. The selected strains were S15 and W15 resist high stress of 1200 µg/ml of chromium and copper when

supplemented in the medium. Secondary screening involved the identification of two isolates by the means of various biochemical tests as shown in table 1. Biochemical characteristics of S15 and W15 strains showed that they belong to the genus *Microbacterium* and *Pseudomonas*, respectively. Palanivel et al., (2020) reported Cu (II) resistant *Pseudomonas stutzeri* LA3 bacterial strain from copper contaminated soil. In another study, Cr (VI) resistant *P. fluorescens* (YPS3) isolated from polluted soil can reduce Cr (VI) from polluted sites (Kalaimurugan et al., 2020). Rajaram et al., (2013) also isolated copper resistant *Microbacterium* sp. (CURB) from contaminated areas.

Table 1: Morphological and biochemical properties of Cr (VI) resistant bacteria isolated from Naran valley.

Characters	Bacterial strains	
	S15	W15
Colony Color	Transparent	Greenish Yellow
Colony Morphology	Small, entire & circular	Large, irregular & circular
Gram Reaction	Positive	Negative
Cell Morphology	Rods	Rods
Motility	+	+
Catalase	+	+
Oxidase	+	+
MR/VP	+/-	-/-
OF Glucose	Aerobic	Oxidative
Indole	-	-
Mannitol Fermentation	-	+

+ = positive, - = negative

Taxonomic Classification of Selected Bacteria

Two Cr (VI) resistant strains selected from thirty-two strains were comparatively analyzed by the 16S rRNA gene sequencing and submitted in the NCBI nucleotide sequence database. The NCBI BLAST was used to match the sequences with the online database (Ye et al., 2006). The strain S15 showed 99 % homology with *Microbacterium* sp. (KX781211). Bacterial strain W15 revealed 99 % homology with the *Pseudomonas* sp. (MT027001) (Figure 2). The percentage of replicating trees revealed that the connected taxi clusters are organized in the bootstrap method (1000 replicates). The neighbor-joining method was used to construct the tree having the scale of branch length with similar units as those of evolutionary distances applied to analyze the phylogenetic tree (Kalaimurugan et al., 2020).

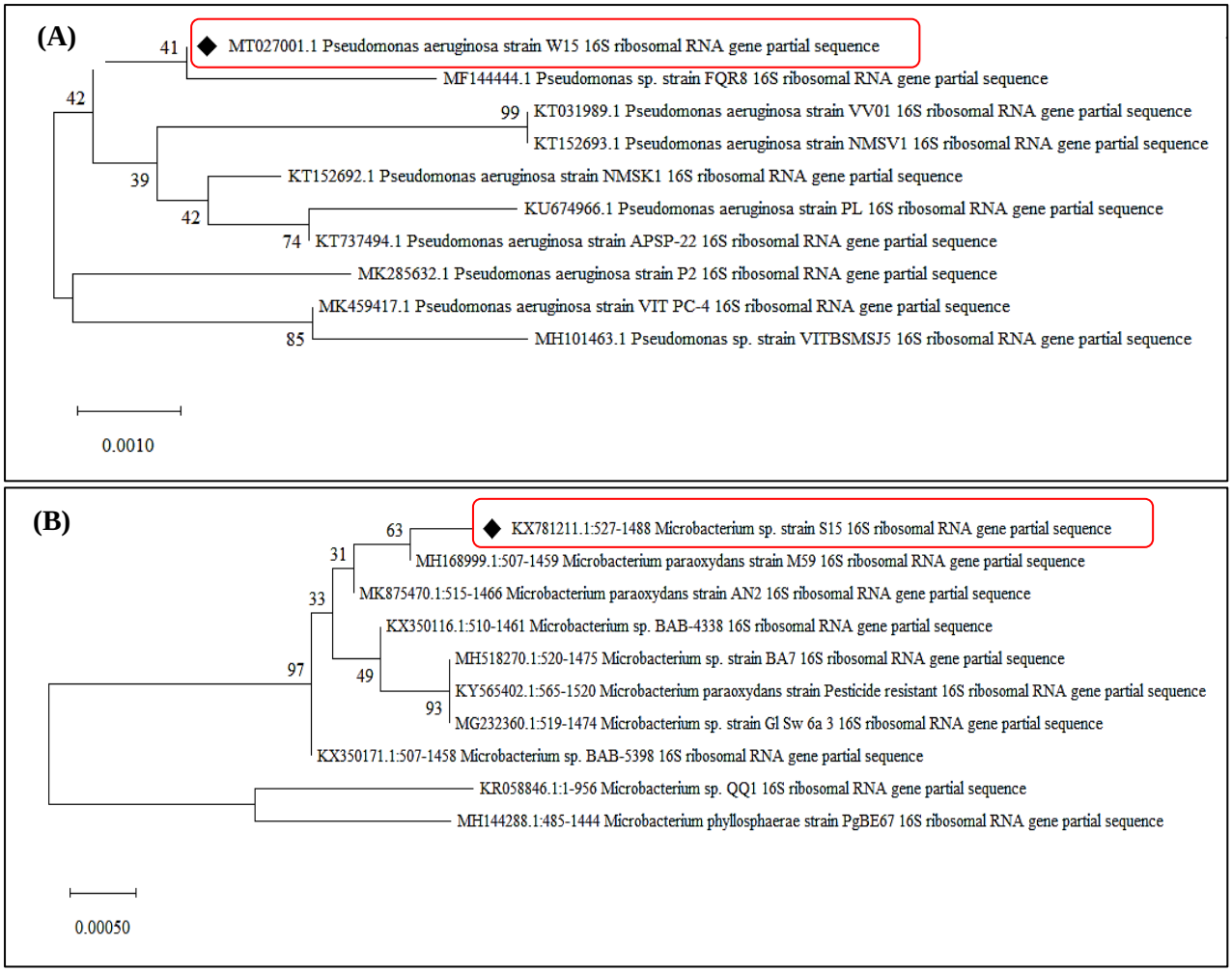

Figure 2: Phylogenetic tree analysis of 16S rRNA gene sequencing of bacterial strains (A) W15, (B) S15. The neighbor-joining method was used to construct these trees. Numbers at nodes represent percentages of 1,000 bootstrap replications (Kalaimurugan et al., 2020).

Determination of MIC of Metal Resistant Bacteria

The growth of S15 and W15 was inhibited at a concentration greater than 1000 µg/ml of zinc and nickel stress however, they revealed consistent growth in the presence of chromium and copper stress up to 1200 µg/ml (Table 2). Vullo et al., (2008) stated the reason for the bacterial tolerance to both copper and chromium is due to the selective pressure and growth in culture conditions that produce various metal binding cations. Against cobalt and nickel stress, both the strains showed less tolerance due to the low pressure of environmental selection. The environmental selection pressure merely depends on how much concentration of heavy metal lies in the log phase (Vullo et al., 2008). Caille et al., in 2007 stated that during the presence of zinc and copper the homeostasis of bacterial cells is tightly regulated since the excess of metal could be toxic. The active efflux of metal cations plays a key role in metal resistance (Caille et al., 2007).

Table 2: Resistance and sensitivity of heavy metal resistant bacterial strains to the various concentrations of certain (A) heavy metals and (B) antibiotics.

(A)	Heavy metal concentration (µg/ml)						
	200	400	600	800	1000	1200	1400
Bacterial strain S15							
Cobalt	+	+	+	-	-	-	-
Nickle	+	+	+	+	-	-	-
Zinc	+	+	+	+	-	-	-
Copper	+	+	+	+	+	+	-
Chromium	+	+	+	+	+	+	-
Bacterial strain W15							
Cobalt	+	+	+	-	-	-	-
Nickle	+	+	+	+	-	-	-
Zinc	+	+	+	+	+	-	-
Copper	+	+	+	+	+	+	-
Chromium	+	+	+	+	+	+	-

(B)	Antibiotic concentration (µg/ml)					
	20	30	40	50	60	70
Bacterial strain S15						
Tetracycline	+	+	+	-	-	-
Erythromycin	+	+	+	+	+	-
Ampicillin	+	+	+	+	+	-
Chloramphenicol	+	+	+	+	+	-
Bacterial strain W15						
Tetracycline	+	+	+	+	-	-
Erythromycin	+	+	+	+	-	-
Ampicillin	+	+	+	+	+	-
Chloramphenicol	+	+	+	+	-	-

+ = positive, - = negative

Chromate Removal Potential of Selected Bacteria

Both of the multiple heavy metal resistant bacterial strains W15 and S15 were studied for chromate removal potential. Bacterial strain W15 exhibited 90% whereas S15 exhibited 80% chromium removal at an initial concentration of 800 µg/ml of chromate within 48 h of incubation (Figure 3). Kumar and Saini (2019) in their study observed the reduction potential of chromate by *Microbacterium* sp. M5. They stated that 100 % reduction was obtained when 200 and 400 µg/ml chromate stress was supplied after 24 and 48 h of incubation, respectively. They also studied M5 cells growing in a medium supplied with chromium 600, 800, 1000, and 1600 µg/ml and resulting in 48.66, 39.75, 32.7, and 16.25% chromate removal after 96 h incubation (Kumar and Saini 2019). In another report by Kang et al., (2017) described *P. aeruginosa* (AB93066)

growing in N-broth achieving Cr (VI) reduction of 62.81 % with chromate stress (11 mg/ml) after 48 h incubation.

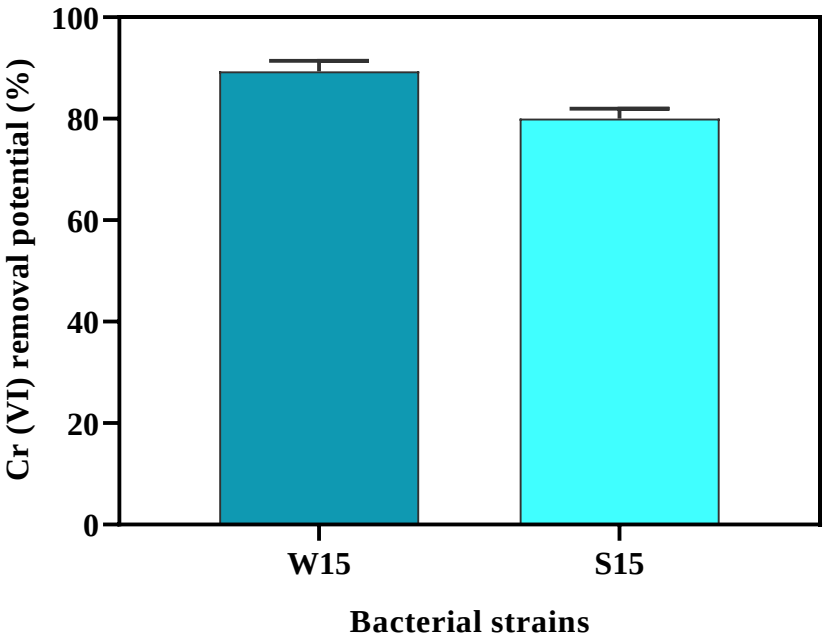

Figure 3: Chromate removal potential of isolated bacterial strains.

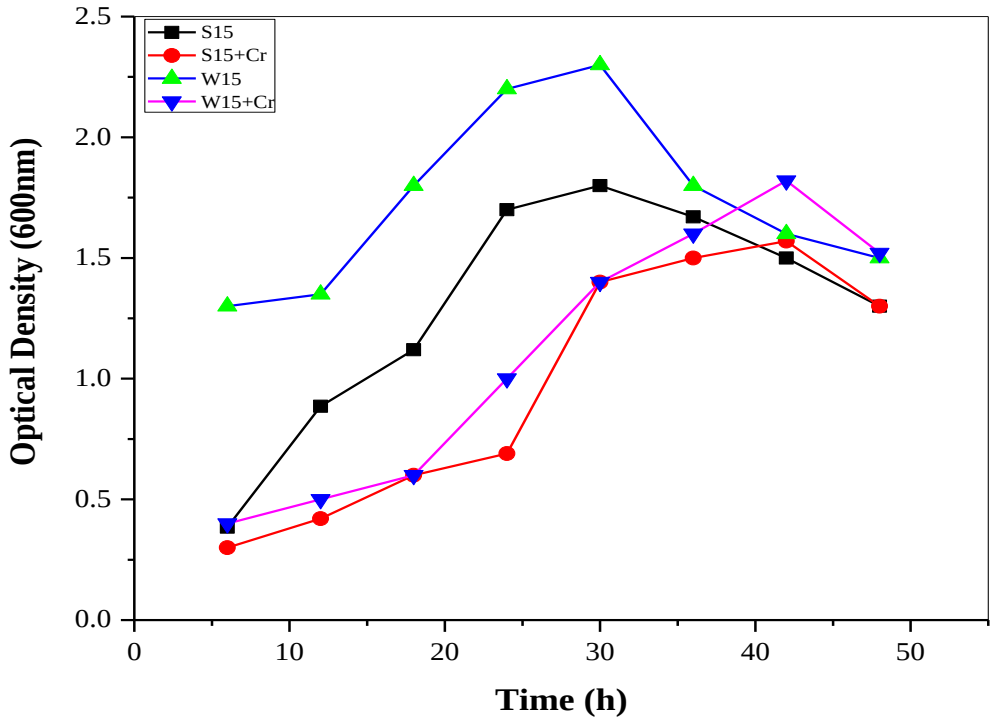

Figure 4: Inductive behavior of Cr (VI) resistant bacteria under chromate stress (1200 µg/ml).

Determination of Inductive Behavior of Selected Bacteria

Both the bacterial isolates were allowed to grow with and without the chromium stress (1200 µg/ml) at 37°C for 48 h (Figure 4). It was observed that the growth pattern of bacterial strains S15 and W15 demonstrated a consistent slower growth (lag phase) when subjected to Cr (VI) stress in comparison to the control conditions. Both strains showed favorable growth at 37°C and pH 7.0. After 30 h of incubation, maximum growth was attained. These results indicated that under chromate stress both the strains showed inductive behavior rather than constitutive. These results were similar to a report by Batool et al., (2012) describing the resistance mechanism of *Ocrobactrum intermedium* to Cr (VI) revealed a lag phase, suggesting that the pathway for Cr (VI) tolerance was inductive. Another report also indicated an inductive pathway for Cr (VI) tolerance for bacterial strain DM1 (Thacker and Madamwar 2005).

CONCLUSION

The present study elucidated the enhanced chromium (VI) removal ability of multiple heavy metal resistant bacterial strains isolated from Naran Valley. Bacterial strains *Pseudomonas* sp. and *Microbacterium* sp. were proficient to remove chromate (90 %) at an initial concentration of 800 µg/ml of Cr (VI). Bacterial resistance to chromium (VI) was inductive when the stimulus (chromium stress) was applied only then did bacteria start synthesizing enzymes/proteins for chromate removal. These strains also exhibited tolerance to other heavy metals suggesting its possible role in remediating the metal contaminated sites.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abou-Shanab RA, Ghanem K, Ghanem N, Al-Kolaibe A (2008). The role of bacteria on heavy-metal extraction and uptake by plants growing on multi-metal-contaminated soils. World J Microbiol Biotechnol., 24(2): 253-262.

Arora M, Kiran B, Rani S, Rani A, Kaur B, Mittal N (2008). Heavy metal accumulation in vegetables irrigated with water from different sources. Food Chem., 111(4): 811-815.

Batool R, Yrjälä K, Hasnain S (2012). Hexavalent chromium reduction by bacteria from tannery effluent. J Microbiol Biotechnol., 22(4): 547-554.

Boone DR, Castenholz RW (2001). Bergey's Manual of Determinative Bacteriology, 2nd Edn, Vol. 1.

Boularbah A, Schwartz C, Bitton G, Aboudrar W, Ouhammou A, Morel JL (2006). Heavy metal contamination from mining sites in South Morocco: 2. Assessment of metal accumulation and toxicity in plants. Chemosphere., 63(5): 811-817.

Caille O, Rossier C, Perron K (2007). A copper-activated two-component system interacts with zinc and imipenem resistance in Pseudomonas aeruginosa. J Bacteriol., 189(13): 4561-4568.

Chary NS, Kamala CT, Raj DSS (2008). Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. Ecotoxicol Environ Safe., 69(3): 513-524.

Freitas H, Prasad MNV, Pratas J (2004). Plant community tolerant to trace elements growing on the degraded soils of Sao Domingos mine in the south east of Portugal: environmental implications. Environ Int., 30(1): 65-72.

Ivezić V, Lončarić Z, Engler M, Kerovec D, Singh BR (2013). Comparison of different extraction methods representing available and total concentrations of Cd, Cu, Fe, Mn and Zn in soil. Poljoprivreda., 19(1): 53-58.

Jenkins SG, Schuetz AN (2012). Current concepts in laboratory testing to guide antimicrobial therapy. Paper presented at the Mayo Clinic Proceedings. Elsevier., 87(3): 290-308).

Kalaimurugan D, Balamuralikrishnan B, Durairaj K, Vasudhevan P, Shivakumar MS, Kaul T, Chang SW, Ravindran B, Venkatesan S (2020). Isolation and characterization of heavy-metal-resistant bacteria and their applications in environmental bioremediation. Int J Environ Sci Technol., 17(3): 1455-1462.

Kang C, Wu P, Li L, Yu L, Ruan B, Gong B, Zhu N (2017). Cr (VI) reduction and Cr (III) immobilization by resting cells of Pseudomonas aeruginosa CCTCC AB93066: spectroscopic, microscopic, and mass balance analysis. Environ. Sci. Pollu. Res. 24(6): 5949-5963.

Katoh K, Standley DM (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol., 30(4): 772-780.

Khan SM, Page S, Ahmad H, Harper D (2012). Anthropogenic influences on the natural ecosystem of the Naran Valley in the western Himalayas. Pak J Bot., 44: 231-238.

Khatri N, Tyagi S (2015). Influences of natural and anthropogenic factors on surface and groundwater quality in rural and urban areas. Front Life Sci., 8(1): 23-39.

Kumar M, Saini HS (2019). Reduction of hexavalent chromium (VI) by indigenous alkaliphilic and halotolerant Microbacterium sp. M5: comparative studies under growth and nongrowth conditions. J Appl Microbiol., 127(4): 1057-1068.

Lokeshwari H, Chandrappa GT (2006). Impact of heavy metal contamination of Bellandur Lake on soil and cultivated vegetation. Curr Sci., 622-627.

Loutfy N, Fuerhacker M, Tundo P, Raccanelli S, El Dien AG, Ahmed MT (2006). Dietary intake of dioxins and dioxin-like PCBs, due to the consumption of dairy products, fish/seafood and meat from Ismailia city, Egypt. Sci Tot Environ., 370(1): 1-8.

Nicholson FA, Smith SR, Alloway BJ, Carlton-Smith C, Chambers BJ (2003). An inventory of heavy metals inputs to agricultural soils in England and Wales. Sci Tot Environ., 311(1-3): 205-219.

Palanivel TM, Sivakumar N, Al-Ansari A, Victor R (2020). Bioremediation of copper by active cells of Pseudomonas stutzeri LA3 isolated from an abandoned copper mine soil. J Environ Manage., 253: 109706.

Rajaram R, Banu JS, Mathivanan K (2013). Biosorption of Cu (II) ions by indigenous copper-resistant bacteria isolated from polluted coastal environment. Toxicol Environ Chem., 95(4): 590-604.

Sadiq MS, Haider S, Abbas G (2005). Genetic parameters for economic traits in exotic germplasm of mungbean (Vigna radiata L. Wilczek). J Agric Res., 43(2): 95-102.

Sato T, Qadir M, Yamamoto S, Endo T, Zahoor A (2013). Global, regional, and country level need for data on wastewater generation, treatment, and use. Agric Water Manag., 130: 1-13.

Simeonov V, Stratis JA, Samara C, Zachariadis G, Voutsa D, Anthemidis A, Sofoniou M Kouimtzis T (2003). Assessment of the surface water quality in Northern Greece. Water Res., 37(17): 4119-4124.

Thacker U, Madamwar D (2005). Reduction of toxic chromium and partial localization of chromium reductase activity in bacterial isolate DM1. World J Microbiol Biotechnol., 21(6): 891-899.

Vullo DL, Ceretti HM, Daniel MA, Ramírez SA, Zalts A (2008). Cadmium, zinc and copper biosorption mediated by Pseudomonas veronii 2E. Bioresour Technol., 99(13): 5574-5581.

Ye J, McGinnis S, Madden TL (2006). BLAST: improvements for better sequence analysis. Nucleic acids research, 34(suppl_2), W6-W9.

Zahoor A, Rehman A (2009). Isolation of Cr (VI) reducing bacteria from industrial effluents and their potential use in bioremediation of chromium containing wastewater. J Environ Sci., 21(6): 814-820.

Zhao K, Liu X, Xu J, Selim HM (2010). Heavy metal contaminations in a soil–rice system: identification of spatial dependence in relation to soil properties of paddy fields. J Hazard Mater., 181(1-3): 778-787.

