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Research Article

Hexavalent Chromium Detoxification and Bioremediation by *Bacillus sp.* from Tannery Effluents

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Abstract: This study examined the Cr(VI) absorption mechanism in indigenous Cr(VI)-tolerant bacterial strains. Five potential chromium-resistant bacterial strains were isolated indigenously from tannery effluent later identified as *Bacillus* sp. using phenotypic and genotypic techniques. In nutrient-rich media, Nutrient Agar, the concentration of Cr was analyzed for maximum tolerance (100 - 1500 µg/ml), and found that the strains showed growth even at 1500 µg/ml. Diphenylcarbazide (DPC) assay was performed to analyze the ability of chromium-reducing bacteria to reduce Cr under various conditions, such as pH, temperature, Cr(VI) content, incubation time, and inhibitors such as antibiotics and heavy metals (Ag, Ni, Zn, Mn and Co). In pilot research, *Bacillus licheniformis* (YAK4) and *Bacillus endophyticus* (YAK7), removed up to 95% Cr(VI) from tannery wastewater in 8 days. The obtained microbial-cleansed water was used afterward in a pot experiment to grow *Vigna radiata* and proved to be useful for the growth of plants. Capacitive heavy metal tolerance and Cr(VI) reduction potentials makes *Bacillus licheniformis* and *Bacillus endophyticus* an ideal option for decontaminating a Cr(VI) contaminated environment.

Keywords: Hexavalent Chromium Reduction, *Bacillus sp*., Tannery Effluent, Chromate Bioremediation, Chromate Resistant Bacteria (CRB), *Vigna radiata*.

1. INTRODUCTION

Chromium is a prevalent man-made pollutant found in soil, groundwater, and surface water. It is utilized in diverse industrial processes, such as metallurgy, chemical production, refractories, tanneries, and wood processing, and many more [1]. Common examples of heavy metals are cadmium, lead, chromium, copper, and extremely poisonous nickel which are found in industrial effluents [2]. These anthropogenic chemicals contaminate the soil, groundwater, sediments, and surface waterways also, including ecological and biological systems. Chromium plays an essential role in the growth of many organisms; however, it is poisonous, carcinogenic, and teratogenic at high concentrations [3]. The US EPA has listed chromium as a priority contaminant [4]. The effluents released by industries possessed Cr(III) and Cr(VI) ranges from ten to hundreds of mg/l. While the existence of nine valence states ranging from +2 to +6, hence, two distinct forms of chromium, namely Cr(III) and Cr(VI), have considerable importance [5]. Since the chromate anion shares structural similarities with SO_4^{2-} and is extremely soluble, it may pass the cellular permeability barrier and enter via sulphate transport channels [6]. The hexavalent form of chromium has a relatively high solubility in water and is thus reported to be a cause of lung cancer, kidney damage, ulcer, and many more [5]. The World Health Organization has category one carcinogen status for Cr(VI) (WHO). The drinking water guideline sets the maximum permitted chromium content in drinking water at 50 ug/l [5]. There exists an imminent need to implement rigorous environmental restrictions aimed at restricting the discharge of Cr(VI) into the environment, with the objective of limiting the

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adverse impacts of Cr(VI) on human health. [7]. Advanced treatment technologies like as reverse osmosis, ion exchange, membrane filtration, electrocoagulation, and electrodialysis have demonstrated efficacy in the removal of Cr(VI). However, they are costly and generate wastes that must be processed and disposed of later. [8, 9]. Hence, bioremediation is developing as a potential approach for removing Cr(VI) from industrial effluents. Several types of fungi and bacteria have been found to remove chromium [10]. Biological remediation techniques use bacteria that can tolerate Cr(VI) and provide cost-effective and environment friendly ways to remove Cr(VI) from contaminated areas [11]. Numerous researchers have investigated the capability of chromium-reducing bacteria to convert Cr(VI) into less hazardous forms. Microorganisms can tolerate the metals in their particular environment. These systems operate inside and outside of cells, lowering the amount of bioavailable metal present in the cell's surroundings [12]. Another protective method involves the expression of exporter proteins, which serve to regulate the metal concentration within the cell to a tolerable level. [13]. The enzymes synthesized by bacteria are diverse kinds and produced by different methods. For instance, intracellular or external membrane-bound reductases are metabolized by bacteria during the direct remediation process [14]. However, oxidants and reductants are generated by indirect mechanisms [15].

Serious health concerns have been raised by chromium emissions that are anthropogenic. According to Lauwerys *et al*. [16], Cr(VI) infusion in the human food chain can cause a number of physiological dysfunctions in humans, including irritability, respiratory infections, and certain allergies. Industrial effluents containing Cr(VI) infiltrate into the soil through the water bodies. As a result, it eventually makes its way into animals' food chains [16]. According to the United States Environmental Protection Agency, Cr(VI) is one of 17 substances that are dangerous to human health, and its allowable range in drinking water has been set at 0.05 mg/l [17]. Bacteria that are capable of tolerating Cr(VI) are utilized in biological remediation techniques and offer cost-effective and environmentally benign solutions for Cr(VI) detoxification from contaminated sites [18]. Thus, the objectives of this study were to (i) separate and identify new Cr(VI)-reducing bacteria from Cr(VI)-contaminated water, (ii) analyze the chromium reduction performance of bacteria, (iii) analyze the ability of bacterial species to degrade hexavalent chromium in the presence of inhibitory salts, heavy metals, and antibiotics, and (iv) determine the hexavalent chromium reduction in industrial wastewater.

2. MATERIALS AND METHODS

2.1. Isolation of Bacterial Strains and Culture Conditions

Five bacterial strains were isolated from the chromium-contaminated tannery effluent and identified based on their morphological characteristics, biochemical tests, physiological, and 16S RNA sequencing. These strains were analyzed for physicochemical properties and were streaked on nutrient agar medium (0.5 g Beef extract, 2.5 g Peptone, 2.5g NaCl, 1 g Yeast extract, and 7.5 g Agar into 500 ml distilled water). They were further checked for their minimum inhibitory concentration (MIC) at different concentrations of potassium dichromate ($K_2Cr_2O_7$) ranging from 100 µg/ml to 1500 µg/ml. All strains were incubated at 37 ℃ for 24 hrs. (pH 7). The results were noted, and strains were stored for a week at 4 ℃ for further testing.

2.2. Physiological Characterization

2.2.1. Temperature influence on bacterial growth

The optimal growth temperature for the isolated strains was identified by subjecting them to varying temperature conditions i.e. 28 °C, 37 °C, and 42 ℃ and incubating them for 24 hrs. The growth characteristics of the strains were examined under both chromate (Cr(VI)) stress conditions alongside standard conditions (without stress), and the optical density was measured at a wavelength of 540 nm. Optical density (OD) indicates the amount of bacteria present in a suspension. The density of bacteria in colony-forming units (CFU) is measured through a spectrophotometer.

2.2.2. pH influence on bacterial growth

Five flasks of nutrient broth for this experiment were prepared and maintained at five different pH levels: 3, 5, 7, 9, and 11. Afterward, 50 µl of each

strain was added to tubes and incubated for 24 hrs. at 37 ℃. To check the bacterial growth, optical density (OD) was measured at 540 nm.

2.2.3. Metals influence on bacterial growth

Various metal resistance profiles, including Ag ($AgNO_3$), Zn ($ZnSO_4$), Mn ($MnSO_4$), Ni ($NiSO_4$), and Co ($CoCl_2$) were determined. For this objective, stock solutions of various metals were prepared by inoculating 1g of metal into 10 ml of distilled water. The nutrient broth was individually poured into tubes. For testing the resistivity of strains, two different metal concentrations: 100 µl/10 ml of broth and 400 µl/10 ml of broth were employed. The findings were evaluated after 24 and 48 hrs. at a temperature of 37 °C.

2.3. Chromium ($K_2Cr_2O_7$) Reduction Experiments

2.3.1. Chromium content determination

All the chromium reduction experiments were carried out in chromium-reducing broth (Tryptone 10 g, Yeast extract 5 g, NaCl 5 g, Citric acid 1 g, and Na_2HPO_4 6.9 g) and under aerobic conditions [19]. Hence, to measure the chromium reduction potential, the medium was supplemented with $K_2Cr_2O_7$ at a 200 µg/ml concentration. An individual test tube was filled with the bacterial culture that was prepared in nutrient broth beforehand. Each tube has 50 µl of bacterial culture, 10 ml of medium, and Cr(VI) as the stated concentration. Subsequently, the tubes were individually subjected to incubation for a duration of four days at a temperature of 37 °C. One milliliter of the bacterial culture was obtained after 24, 48, and 72 hrs. and centrifuged for five minutes at 12000 rpm, centrifugation was employed to get the bacterial pellet. Diphenylcarbazide (DPC) spectrophotometric technique was used to track Cr(VI) reduction [20]. In this experimental procedure, a volume of 100 µl of the supernatant was transferred from the Eppendorf tube to a test tube containing 10 ml of distilled water. The test tube was then filled with 1 ml of 0.5% of Diphenylcarbazide (0.5 g of 1,5-diphenylcarbazide, 100 ml of acetone) and a few drops of H_2SO_4. The tube was allowed to remain at room temperature for the next 15 to 20 minutes or until the color becomes purple [21]. At a wavelength of 540 nanometers, the optical density in the solution was measured. Based on the results obtained from the calibration curve, $K_2Cr_2O_7$ reduction was carried out. The percentage of chromium reduction was determined by calculating the final Cr(VI) concentration found in the medium using a standard curve that includes both the treatment and the control [20].

$$Cr(VI) reduction\ (\%) = \frac{C^i - C^f}{C^i} \times 100$$

where,
C^i = Initial $K_2Cr_2O_7$ concentration
C^f = Final $K_2Cr_2O_7$ concentration

2.3.2. Chromium reduction and effect of pH

In test tubes, chromium reduction media was prepared. We used five test tubes for each strain, with pH values of 3, 5, 7, 9, and 11. We added a 250 µg/ml supplement to the medium before administering the strains. The tubes were then kept in an incubator at 37 °C. At 540 nm, the optical density was measured after 24 and 48 hours, respectively, and a graph was drawn on the calibration curve to determine the amount of chromium present.

2.3.3. Chromium reduction and effect of temperature

In test tubes containing chromium reduction broth and 250 µg/ml $K_2Cr_2O_7$, we performed the experiment at three different temperatures, 28 °C, 37 °C, and 48 °C. In light of the findings of the earlier tests, the pH was settled (at optimum pH). The strains were added into the tubes and incubated for 24 and 48 hours. Following that, the optical density at 540 nm was measured to estimate the chromium concentration, and a graph was drawn in accordance with it.

2.3.4. Synergistic effect of pH and temperature on chromate reduction

The impact of Cr(VI) addition was examined in this study, coupled with the synergistic effect of varying temperature and pH for each strain. For this, chromate reducing broth was prepared by maintaining various pH levels (3, 5, 7, 9, and 11). The strains were incubated at different temperatures of 30 ℃, 37 ℃, and 48 ℃. $K_2Cr_2O_7$ was added to the medium at two distinct concentrations: 200 µg/ml and 400 µg/ ml for 24 and 48 hours, respectively.

A negative control was also placed alongside the other strains. The DPC test was then performed for a large number of samples in a microtiter plate. To bring the volume of the solution to 200 µl, 160 µl of water, 4 µl of bacterial inoculum, 20 µl of DPC, and about 2 µl of H_2SO_4 were added. Microtiter plate was allowed to rest for 15 - 20 minutes. At 540 nm, the optical density was measured. This experiment allowed us to determine the hexavalent chromium reduction by bacterial strains at optimum pH as well as temperature and analyze the effect synergistically.

2.4. Antibiotic Disk Diffusion Assay

Heavy metal resistance genes and antibiotic resistance genes are frequently found together on plasmids and can be utilized for further genetic manipulation studies of the existing resistance characteristic traits [22]. This experiment provides information about the presence of antibiotic resistance traits in bacterial strains. Muller Hilton agar was prepared, and 4 antibiotics (Erythromycin (15 µg), Neomycin (30 µg), Tetracycline (10 µg), and Ampicillin (5 µg) of above stated concentrations were taken and administered for each strain. After the 18-24 hours' incubation, a measuring scale was used to measure the inhibition zones for each antibiotic disc and mentioned in mm [23]. Afterward, the noted zones were compared with CLSI, 2020.

2.5. Determination of Chromium Reduction in Tannery Effluent

For this experiment, 250 ml of nutrient broth was inoculated with 50 µl of each strain; the OD was taken and then incubated for 7 days. After measuring the optical density of the broth, we added 50 ml of the broth from each flask into 500 ml of tannery water. We let it incubate for 10 days on the shaker at room temperature. The control was also placed alongside and the samples were extracted at regular intervals, specifically at 2, 4, 6, 8, and 10 days of incubation to monitor the chromium reduction by measuring the optical density. After 10 days of incubation, the final readings were taken and found considerable chromium reduction with the help of the Diphenylcarbazide method [24].

2.6. Microbial Treated Wastewater and Pot Experiment

Microbial-treated wastewater was used to cultivate plants. For this, we prepared small pots, and mung beans (*Vigna radiata*) were sown in each pot. Tap water was used for the control pot, while treated wastewater (5 ml) was given to the experimental plants. Seeds were allowed to grow for 7 days to determine the effect of chromium metal on plant growth. The seed germination and other changes were observed.

3. RESULTS

3.1. Bacterial Strains and Culture Conditions

Strains were extracted from sewage water at a tannery, and after morphological analysis and gram staining, we found that they were all gram-positive rods and positive for spore stain (Table 1). All strains showed positive growth till 1500 µg/ml of $K_2Cr_2O_7$. The strains were characterized and identified by 16S rRNA sequencing. Blast was performed on obtained sequence for each strain and accession number was assigned to them accordingly. These strains were identified as: YAK-1 *Bacillus foraminis*, YAK-2 *Bacillus thuringiensis*, YAK-4 *Bacillus licheniformis*, YAK-7 and YAM *Bacillus endophyticus*.

3.2. Comparative Analysis of pH and Temperature of Bacterial Isolates

To analyze the bacterial growth at varying pH, temperature, and time, the media was inoculated

Table 1. Biochemical analysis of the strains.

Isolates	Oxidase test	Starch hydrolysis	Indole test	VP	MR	Urease
YAK 1	+	+	-	-	+	-
YAK 2	-	+	-	+	-	-
YAK 4	+	+	-	+	+	-
YAK 7	-	-	-	-	+	-
YAM	+	-	-	-	+	-

with fresh cultures of the isolates. Five tubes from each set were incubated at three different temperatures: 28 °C, 37 °C, and 48 °C. At 37°C, all strains exhibited good growth, with YAK4 and YAM showing exponential growth, achieving OD values of 1.4 and 1.9, respectively (Figure 1A). Regarding different pH levels (3, 5, 7, 9, and 11), all strains demonstrated steady growth at pH 7. However, YAK4 performed particularly well at pH 5 and 9, with OD values of 1.2 and 1.09, respectively. In contrast, YAK1 and YAK2 showed optimal growth at pH 9, with OD values of 1.02 and 1.08 (Figure 1B). After 72 hours of incubation, all strains exhibited significant growth, with YAK7 and YAM displaying the best growth, with OD values of 2.0 and 1.76. In conclusion, the optimal conditions for growth were found to be 37°C and pH 7, with the highest growth observed after 72 hours (Figure 1C)

3.3. Heavy Metals Resistance Profile

In addition to chromium, five other heavy metals were tested for and employed for isolated strains. To test the strains' tolerance to metals, the strains were exposed to two distinct metal concentrations: 100 µg/ml and 400 µg/ml. The findings after 24 and 48 hours are shown in Figure 2. Optical density for each strain was observed at 540 nm. The results at a concentration of 100 µg/ml showed that YAK4 performed well against $ZnSO_4$, while YAK7 performed well against $MnSO_4$ after 24 hours, with optical densities of 0.519 for YAK4 and 0.521 for YAK7. YAK4 showed good performance against $ZnSO_4$, the OD was 0.491 after the time period of 48 hours, similarly, YAK2 showed significant growth against $MnSO_4$ with OD 0.495 as compared to its performance at 24 hours (Figure 2A). Comparatively, at 400 µg/ml conc. the observed value of OD was 0.294 at which YAK2 worked well under the influence of $AgNO_3$ and OD was 0.371 for YAK7 at which it exhibited effective growth against $MnSO_4$ after 24 hours of incubation. However, only YAK7 demonstrated good growth against $ZnSO_4$ after the incubation of 48 hours (Figure 2B).

3.4. Chromium ($K_2Cr_2O_7$) Reduction Experiments with Bacterial Strains

As the chromium content of the medium is decreased by the bacterial strains, a DPC test was

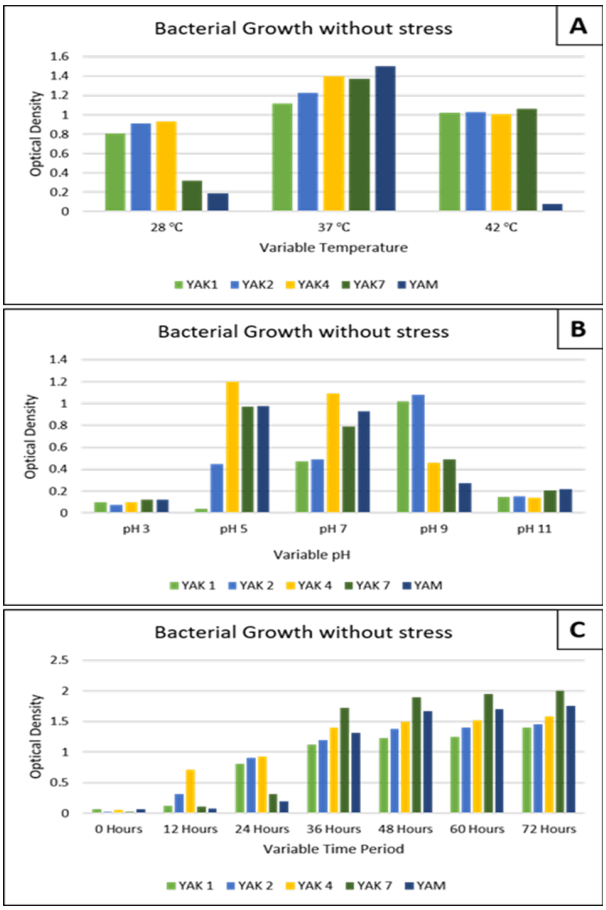

Fig. 1. Bacterial growth at variable temperatures (A), variable pH (B), and at different time intervals of 0 to 72 hrs (C).

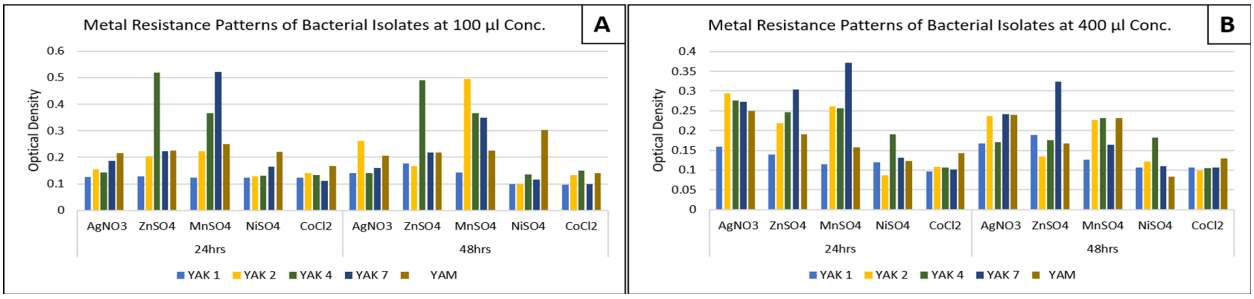

Fig. 2. Bacterial growth analysis and determining the heavy metal tolerance with 100 µg/ml of metal (A) and 400 µg/ml of metal at the time interval of 24 hours and 48 hours (B).

performed to determine its concentration. While the concentration of $K_2Cr_2O_7$ was kept at 200 µg/ml with incubation durations of 24 and 48 hours, respectively, to check for reduction (Figure 3). All the bacterial strains performed well in reduction experiment, however, YAK7 and YAM reduced chromium concentration significantly well while the temperature was kept 37 ℃ at pH 7.

3.5. Analyzing pH, Temperature, and Time in Comparison with $K_2Cr_2O_7$

$K_2Cr_2O_7$ was added as stress and examined the growth of bacteria at various pH levels, temperatures, and time intervals. Two amounts of Cr(VI) to the media—200 µg/ml and 500 µg/ml were employed. Chromate-reducing medium was used in test tubes and varied the values of 3, 5, 7, 9, and 11 for pH. The tubes were incubated at varying temperatures of 28 °C, 37 °C, and 48 °C. We observed bacterial growth after 24 and 48 hours. At 540 nm, the optical density 0.74 was determined and found that YAM exhibited optimal performance at 28 °C, while at OD 0.685, YAK7 performed best at 48 °C, with chromate concentration set at 200 µg/ml for a 24-hour incubation period (Figure 4A). At a concentration of 500 µg/ml, at 37 ℃ all strains showed nominal growth over a period of 24 hours while YAK4 with OD value 0.744 demonstrated exponential growth at 28 °C after 48 hours (Figure 4B). The comparison of bacterial growth at variable pH revealed that at 24 hours YAK1 performed well at pH 5 while YAM showed exponential growth at pH 9 with a concentration of 200 µg/ml and optical density was 0.08 and 0.096 after 48 hours (Figure 5A). In comparison, the OD was 0.285 at which YAK7 demonstrated optimal performance at pH 9 when exposed to a concentration of 500 µg/ml after 48 hours of incubation (Figure 5B).

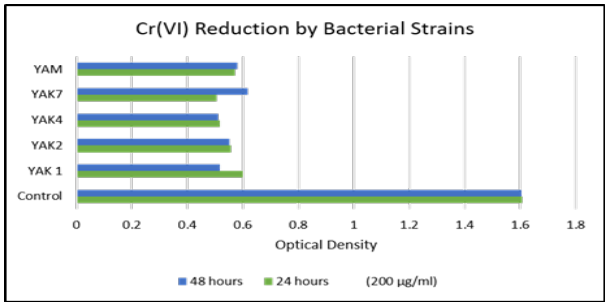

Fig. 3. Reduction of Cr(VI) at 200 µg/ml after 24 hours and 48 hours.

3.6. Chromium Content Determination

The concentration of $K_2Cr_2O_7$ in the media was determined by Diphenylcarbazide Assay (DPC). As the bacterial strains reduced the chromium content, a DPC assay was employed to assess rest of the chromium present in the media. The concentrations of chromium were 200 µg/ml and 500 µg/ml. The temperature differences (30 °C, 37 °C, and 48 °C) and pH levels (3, 5, 7,9, and 11) were used by providing 24 and 48-hour incubation periods, respectively. The outcomes revealed that with OD value 0.948 YAK1 exhibited the best reduction at pH 5 and 37 °C, with the measured reduction potential being 51%. For the strains YAK2 and YAM, the optical density observed was 0.953 and 0.94 at which the reduction was 54% and 60%, respectively (Figure 6).

On the other hand, the ideal pH was found to be pH 9 at 30 °C after 48 hours. With OD 1.749 YAK7 at pH 9 performed well while the temp was 30 °C, and the reduction potential was 78% (Figure 7A). However, at 37 °C, both YAK7 and YAM exhibited good reduction showed OD value 2.30

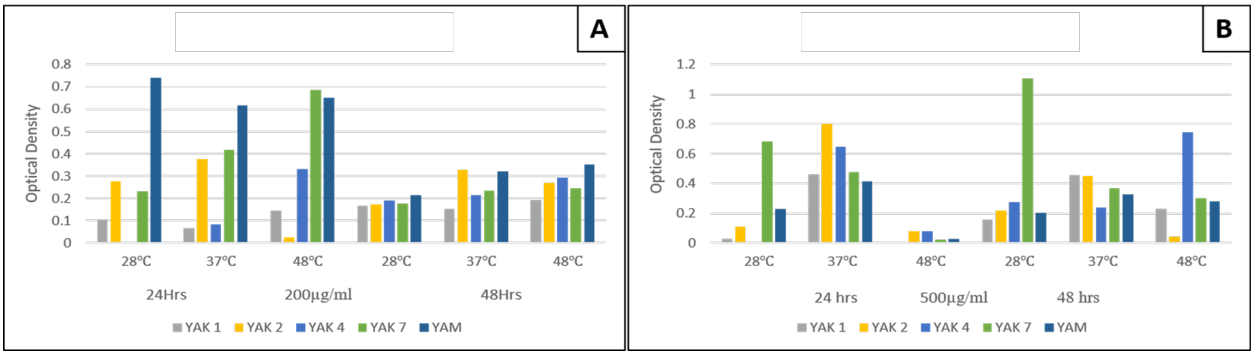

Fig. 4. Comparison of bacterial growth at various temperatures in the presence of different Cr(VI) conc. 200 µg/ml (A) 500 µg/ml (B).

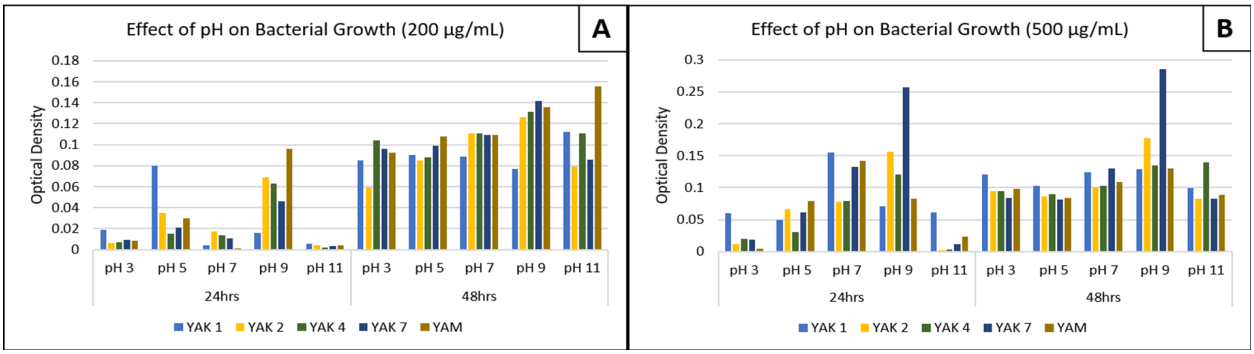

Fig. 5. Comparison of bacterial growth at different pH in the presence of variable Cr(VI) concentrations, 200 µg/ml (A) 500 µg/ml (B).

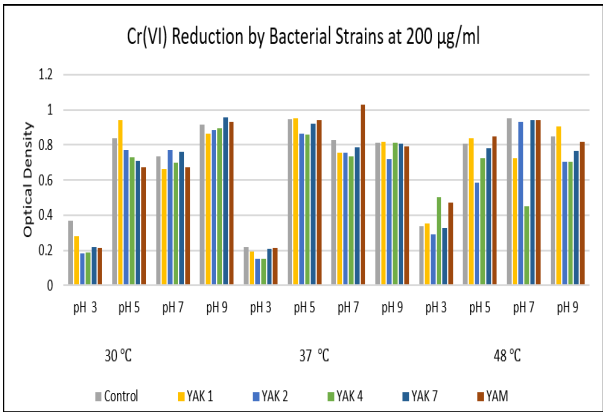

Fig. 6. Cr(VI) Reduction by Bacterial Strains at 200 µg/ml under variable pH at 30 °C, 37 °C, and 48 °C over a 24-hour time period.

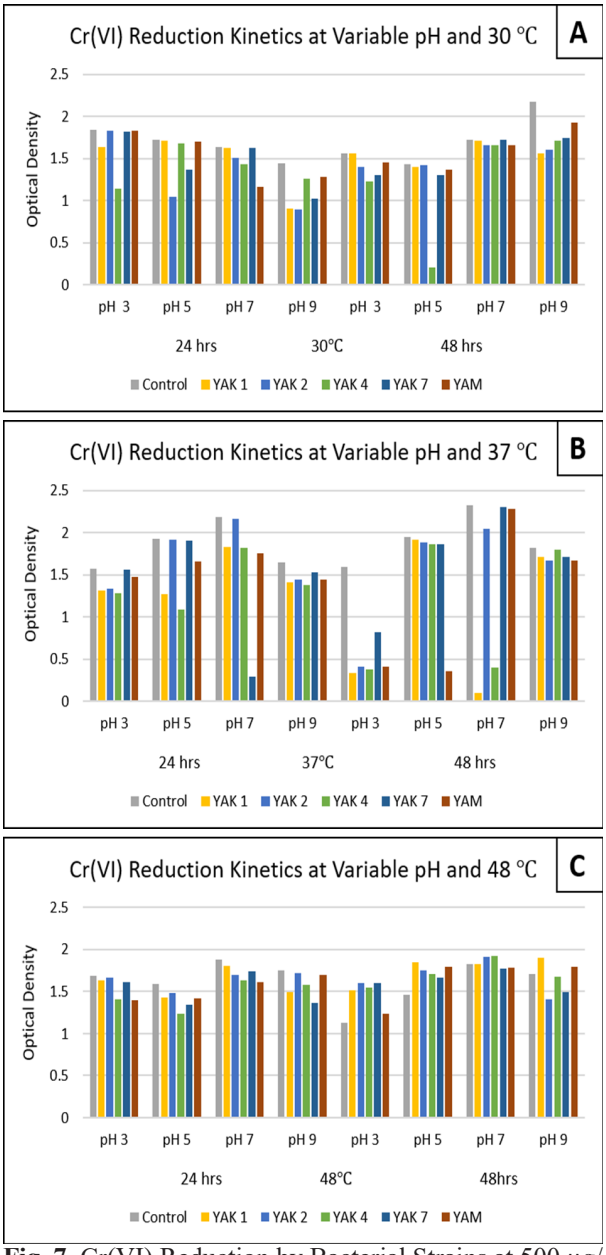

Fig. 7. Cr(VI) Reduction by Bacterial Strains at 500 µg/ml under variable pH at 30 °C (A), 37 °C (B), and 48 °C (C) with 24- and 48-hour time intervals.

and 2.28 at pH 7, with potentials of 77% and 76%, respectively (Figure 7B). However, YAK4 with OD value 1.923 performed great reduction while the pH was 7 at 48 °C, and the reduction potential was 70% (Figure 7C).

3.7. Antibiotic Susceptibility Testing

Four antibiotic discs that were deemed to be more efficient against *Bacillus* species were taken. Muller Hilton agar it was used. Each plate comprised four discs (Figure 8). The zone of inhibitions was seen after the incubation period of 20 to 24 hours. After measurement, it was interpreted that all the strains were sensitive towards neomycin and tetracycline, however, resistance towards ampicillin (Table 2).

3.8. Bioremediation Experiment of Hexavalent Chromium

The efficiency of chromium-reducing strains was determined by supplementing the bacterial

Fig. 8. Antibiotic disk diffusion testing.

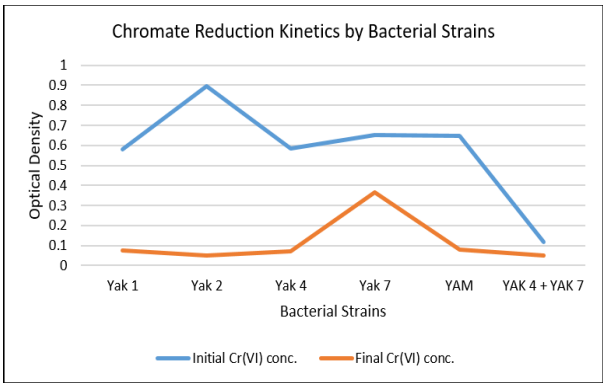

Fig. 9. Chromate reduction by chromium-reducing strains before incubation and 10 days after room-temperature incubation.

Table 2. Antibiotic testing.

Antibiotics	Strains				
	YAK 1	YAK 2	YAK 4	YAK 7	YAM
Erythromycin	R	I	S	I	I
Neomycin	S	S	S	S	S
Tetracycline	S	S	S	S	S
Ampicillin	R	R	R	R	R

Key: R = resistant, I = intermediate, S = sensitive

inoculated broth into the tannery wastewater. After the incubation of 10 days, the chromium reduction was measured by performing a Diphenylcarbazide assay, and a graph was plotted based on the results (Figure 9). YAK4, YAK7, and YAM reduced chromate up to 87%, 67%, and 88% respectively.

3.9. Effect of Treated Wastewater on *Vigna Radiata*

The germination of *Vigna radiata* seeds was tested by using microbial-treated wastewater. Considerable growth of *Vigna radiata* was seen as compared to untreated wastewater. It was observed that after bacterial treatment, the toxicity of the original wastewater significantly decreased along with a decrease in the noxious harmful Cr(VI). After two days of exposure to the microbial-treated water, the germination of the young seedlings was successfully observed (Figure 10A). The transition to the seedling stage (post-germination) occurred after six days of continued watering with the treated solution. To assess the effects of the microbial treatment on seed growth, a control group was maintained, where the mung bean seeds were watered with standard tap water. This allowed for a comparative analysis of seedling development under different conditions (Figure 10B).

4. DISCUSSION

In this era of industrialization, the persistence and inability to degrade heavy metals, bioremediation is a major study topic worldwide [25]. In addition to other chemical agents, some heavy metals also contribute to contemporary concerns. Some metals, such as chromium, copper, cadmium, lead, selenium, and nickel, are natural poison reservoirs and negatively impact biological and ecological ecosystems [26].

The major industrial areas in Punjab are Kasur and Faisalabad, which are home to a number of significant enterprises that have helped the state's economy but have also aggressively polluted the region's otherwise beautiful environment. Due to the inability of heavy metals produced in large quantities by industries to degrade, their discharge into the biosphere has both an immediate and a cumulative impact [27, 28]. These heavy metals are byproducts of several industrial operations, such as leather tanning, wood preservation, and pulp manufacturing. They are discharged into the environment in the form of effluents, and their concentrations vary [25]. Due to their limited solubility in biota and persistent nature as a pollutant, heavy metals pose a significant threat to both human and environmental health. Several of the above-stated heavy metals are categorized as carcinogens and mutagens [29].

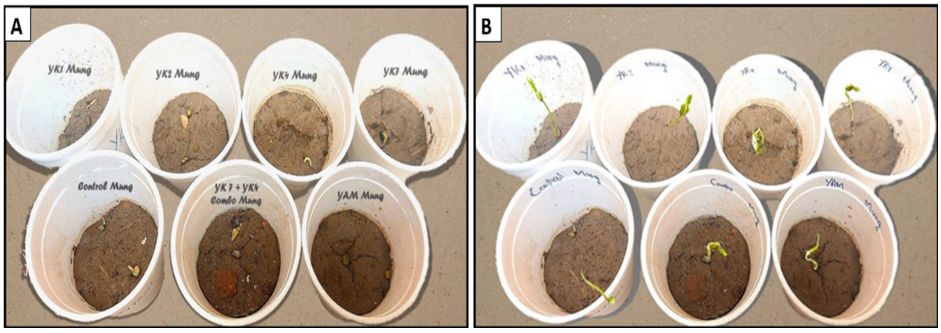

Fig. 10. *Vigna radiata* seed germination after 2 days (A), and *Vigna radiata* growth after 6 days of giving microbial-treated wastewater (B).

Chromium is one of the hazardous heavy metals typically present in industrial effluent. It is also regarded as a necessary micronutrient for the growth of plants and many other species. Yet, when present in larger concentrations, chromium serves as a carcinogen and teratogen [30]. The chromium content in industrial effluents varies depending on the concentration utilized, ranging from 10th to 100th of mg/l in the form of Cr(III) or Cr(VI). Additionally, due to their stability, Cr(III) and Cr(VI)are more significant in the environment [31].

Cr(VI) is one of the primary causes of lung cancer, renal damage, chromate ulcer, and perforation of the nasal septum, according to earlier findings [32]. In addition, it is hazardous to species such as plants and animals that eat water contaminated with chromium. In contrast, the trivalent form of chromium is less harmful and is used by several species as a micronutrient. It is 100 times less hazardous than Cr(VI) in nature and also insoluble in water [33].

A total of 10 isolates that exhibited resistance to chromium were obtained from industrial sites in Faisalabad that had been chronically polluted with chromate. Among these isolates, 5 strains belonging to the *Bacillus* genus were selected for subsequent analysis. Several researchers have already identified and employed *Bacillus* isolates that exhibit resistance to chromate [27, 34-36]. Initial studies indicate that the strains were most likely *Bacillus sp*., since all the bacterial isolates were gram-positive rods capable of producing spores and exhibiting small, spherical, mucoid, irregularly shaped colonies. In a previous study various *Bacillus* species have shown strong chromium tolerance up to > 2500 mg/ml [37].

The bacterial strains were screened for their optimal pH and temperature by giving varying $K_2Cr_2O_7$ concentration, and we discovered that pH 9 was the best pH for growth at 30 °C at 200 µg/ml. After 24 hours of incubation at 48 °C, we observed that pH 7 was effective, while pH 5 was the ideal pH at 37 °C. Tan *et al*. [38] discovered that the pH in the range 6 and 9 was ideal for *Bacillus* species. These results are inline in the present study; however, a slight deviation from the results might be brought on by environmental circumstances. The ideal pH was found to be pH 9 at both temperatures, i.e., 30 °C and 37 °C, when we examined the findings at the concentration of 500 µg/ml. It supports the previous findings that the ideal pH 7 was determined at 48 °C [38, 39].

Due to the abundance of naturally occurring heavy metals in the biosphere, bacteria have resisted them [40]. In a study by Faisal *et al*. [26] explained how some bacterial strains might reduce heavy metals, including Cu, Ag, Se, Co, Cr, Zn, Mn, and Pb. We also assessed the growth potential of our isolated isolates by testing them against various heavy metals and discovered that the strains were effective against $AgNO_3$, $MnSO_4$, and $ZnSO_4$ at 100 µg/ml and 400 µg/ml concentrations. When the growth was assessed after 48 hours, 72 hours, and 168 hours, it was observed that the two strains, YAK4 and YAK7, performed exceptionally well at both concentrations. The current study's findings are consistent with those of [41, 42]. Chromium-resistant strains can also withstand additional heavy metals [33, 43].

Since wastewater lacks the proper nutrients for microbial growth and contains toxic compounds, it works as a particularly unfavorable environment

for the growth of non-native bacteria. In earlier research, there has been a lot of attention given to the evaluation of chromate reduction in lysogeny broth (LB) broth media [34, 44]; however, few studies have performed the bio reduction trials in actual industrial effluents. In this study original tannery effluent was used to perform the bioremediation studies at pilot scale level and examine the strain's ability in nutrient medium. The previously identified bacterial strains exhibit the ability to effectively remove Cr(VI) ions from the initial tannery wastewater. These isolates are therefore suitable to use in bioremediation experiments under real-world conditions. The chromate bio reduction potential of *Staphylococcus capitis* and *Bacillus sp*. JDM-2-1 was also investigated by Zahoor and Rehman [45] in the industrial wastewater. They discovered that both species had the ability to reduce Cr(VI) to Cr(III) by 81% and 85%, respectively. In chromium reduction media, the ability of the isolates was evaluated. After running a DPC test, it was determined that YAK1 exhibited the best reduction at pH 5 and 37 °C, with a reduction potential of 51%. At 30 °C, the ideal pH for the strains YAK2 and YAM was pH 9, with a decrease of 54% and 56%, respectively. For YAK4, pH 7 performed well at 48 °C, and the reduction potential was 76%; for YAK7, pH 9 performed well at 30 °C, and the reduction potential was 78%.

5. CONCLUSIONS

Based on the overall findings, it was determined that the strain YAK4 was identified as *Bacillus licheniformis*, whereas both YAK7 and YAM were identified as *Bacillus endophyticus*. These strains outperformed all others and were able to remove chromium by 75% to 80% when the temperature is 30 °C to 37 °C and pH between 7 and 9. Enhanced culture conditions can improve the reduction potential. We also observed that YAK4 and YAK7 performed well during the pilot scale study (*Vigna radiate* pot experiment). Hence, it was concluded that these strains have a high potential to reduce carcinogenic hexavalent chromium and may be employed in bioremediation applications because of their improved stability, high reduction potential, and ease of reusing.

6. ACKNOWLEDGMENT

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7. CONFLICT OF INTEREST

There are no conflicts of interest declared by the authors.

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