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Isolation And Characterization Of Chromium And Lead Reducing Bacteria For Bioremediation Of Chromium And Lead From Soils And Effluents.

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Abstract

The primary laboratory effort in this work was to improvising the indigenous chromium remediating *Bacillus subtilis* (PESA-JX081251) and lead remediating *Bacillus amyloliquifaciens* (PES B - JX112654) strains by generating random mutations utilizing physical and chemical mutagens employing established methodologies. The best chromium and lead remediation mutants were then immobilized on magnetic iron oxide nanoparticles to speed up the remediation process and lyophilized for easy administration into printing effluents. Treatment with *Bacillus subtilis* and *Bacillus amyloliquifaciens* microorganisms was used in a series of tests to determine chromium and lead remediation (Zhao and Kaluarachch 2002). Some strains of bacteria were isolated from, sludge samples gathered from effluent discharged sites of industrial and municipal wastes via different labelled as BS (Gadd 2010).

Keywords: Bioremediation, Chromium Reducing Bacteria, Lead Reducing Bacteria, Soil, Heavy Metals, Bacillus-mediated remediation.

INTRODUCTION

The samples of soil were taken near Partapur Industrial area, Mohkampur Industrial area, Mawana Road Industrial area and printing press. The samples were gathered from the dyeing business every two weeks for a year, and the work was completed between 2018 and 2021.The results of physiochemical analysis of all the electroplating industrial effluent clearly indicated that samples were having appreciable amount of toxic chromium(VI) and lead (Tyagi *et.al.,* 2018). Further for the bioremediation studies of chromium (VI) and Lead from effluent, chromate reducing bacteria (CRS) and lead reducing bacteria (LRS) were isolated (Singh *et.al.,* 2019).

Some bacterial strains were isolated from, sludge samples collected from effluent discharged sites of industrial and municipal wastes via different labeled as BS. Out of these bacterial strains only 04 bacterial strains have shown chromium (VI) and Lead Toxicity using *Bacillus subtilis* and *Bacillus amyloliquifaciens*microbes' reduction property. Chromium reduction property of isolated bacterial strain was studied by examining chromium (VI) solution samples inoculated by various bacterial strains for incubation period of 48 hours. Examination of chromium (VI) and Lead Toxicity using *Bacillus subtilis* and *Bacillus amyloliquifaciens*microbes' solution has been done by UV-Visible spectrophotometer

Strains Isolation, Maintenance, And Preservation Of Cultures Wild Type Strains Of *B. Subtilis* And *B. Amyloliquifaciens*

To produce the suspension culture, a loopful of culture of *B. subtilis and B. amyloliquifaciens* strains that had been revived on the plate were individually injected in 50 ml of LB broth as well as marked overnight at 37ºC (Verma *et.al.,* 2016). Whenever the absorption of the overnight values would be around 1 OD, 1 ml each of *B.subtilis and B. amyloliquifaciens* were inoculated into Luria Bertani (LB) broth (Hi-Media) pointed by chromium at a absorption of 100 mg / L of hexavalent chromium and lead (sourced from potassium dichromate). The chromium concentration was measured every hour starting from the moment of injection. 1 ml of spiking medium culture was collected, subjected to centrifugation at 4 ºC for 10 minutes at 5000 rpm,

and supernatant were chromium tested using the diphenylcarbazide test. Estimation of chromium and lead was maintained until the levels of chromium and lead were stable. All of the trials were repeated three times.

A loopful of cultured *B. subtilis* and *B.amyloliquifaciens* strains were injected individually in chromium-laden electroplating effluent at a concentration of 490 mg/L. As a nutritional supplement for the microorganisms, 5% sucrose was added to the medium. The chromium and lead concentrations were measured every 24 hours starting from the moment of inoculation. 1 ml of the inoculated effluent culture was extracted, subjected to centrifugation at 4 ºC for 10 minutes at 5000 rpm, and supernatant were chromium tested and lead using the diphenylcarbazide assay and the DMTD method. Estimation of chromium and lead was maintained until the levels of chromium and lead were stable. All of the trials were repeated three times.

Sampling, Isolation and maintenance of Culture

The primary laboratory effort in this research was improvising the indigenous chromium remediating *Bacillus subtilis* (PESA-JX081251) and lead remediating *Bacillus amyloliquifaciens* (PES B - JX112654) strains by generating random mutations utilizing physical and chemical mutagens employing established methodologies. The best chromium and lead remediation mutants were then immobilized on magnetic iron oxide nanoparticles to speed up the remediation process and lyophilized for easy administration into printing effluents. Treatment with *Bacillus subtilis* and *Bacillus amyloliquifaciens* microorganisms was used in a series of tests to determine chromium and lead remediation (Zhao and Kaluarachch 2002).

Isolation of bacterial strain

Some bacterial strains were isolated from, sludge samples collected from effluent discharged sites of industrial and municipal wastes via different labeled as BS(Gadd 2010).

Screening of chromate reducing bacteria

Out of these bacterial strains only 04 bacterial strains have shown chromium (VI) and Lead Toxicity using *Bacillus subtilis* and *Bacillus amyloliquifaciens*microbes' reduction property. Chromium reduction property of isolated bacterial strain was studied by examining chromium (VI) solution samples inoculated by various bacterial strains for incubation period of 48 hours. Examination of chromium (VI) and Lead Toxicity using *Bacillus subtilis* and *Bacillus amyloliquifaciens*microbes' solution has been done by UV-Visible spectrophotometer at Lemda(max)=540nm by using 1,5 diphenyl carbazide (Low 2000).

Table 1: Screening of CRS from stock solution of chromium (VI)

Sr.No	Bacterial Isolates	% Reduction of Cr (VI)	% Reduction of Pb (IV)
1	BS-1	00	00
2	BS-2	67.48	60.59
3	BS-3	00	00
4	BS-4	64.02	62.29
5	BS-5	00	00
6	BS-6	00	00
7	BS-7	61.1	63.13
8	BS-8	62.15	65.17
9	BS-9	00	00

Revival of chromium remediating microbial cultures; *B.subtilis* and *B.amyloliquifaciens*

The glycerol stocks of *B.subtilis* and *B.amyloliquifaciens* were revived. On overnight culturing at 37 ºC on Luria Bertani agar plate, the *B.subtilis* appeared as white fried egg colonies with serrated edges (Fawzy 2008) as indicated in Figure 1. *B.amyloliquifaciens* appeared as off white, smooth mucoid colonies as presented in Figure 2.

Figure 1: Pure culture of *Bacillus subtilis*

Figure 2: Pure culture of *Bacillus amyloliquifaciens*

Table 2: Recoding of chromate reducing strains (CRS) and lead reducing strains (LRS)

Sr.No	**Bacterial strain**	**Colour of colony**	**New Code of bacterial strain**
1	BS-2	White	CRS-W
2	BS-4	Yellow	CRS-Yl
3	BS-7	Pale yellow	LRS-Y2
4	BS-8	Red	LRS-R

CRS- Chromate reducing strain, LRS-lead reducing strains, W-White, Yl-Yellow 1, Y2-Yellow2, R- Red

These isolated bacterial strains only some bacterial strains were found capable for high reduction of toxic chromium (VI) and lead for which we have selected i.e*Bacillus subtilis* and *Bacillus amyloliquifaciens*. Further bioremediation study for chromium (VI) and Lead Toxicity using *Bacillus subtilis* and *Bacillus amyloliquifaciens* microbes has been done by using selected bacterial strains (Tharannum *et.al.*, 2012).

After reviving the glycerol stocks of *B.subtilis and B. amyloliquifaciens*, the chromium and lead removal efficacy of the strains in suspension and immobilized form were tested in chromium and lead spiked samples as well the electroplating effluent samples (Gray JS. 2019).

The chromium and lead reducing ability of the wild type bacteria, that is, *B.subtilis* and *B.amyloliquifaciens* was assessed by inoculating an overnight culture in its log phase (1.8 X 10 ^{9} cells) into media amended with chromium at a known concentration of 100 mg/L respectively. to be 490mg/L. The chromium and lead estimation by DPC assay and 2, 5-dimercapto-1, 3, 4-thiadiazole (DMTD) was carried out for culture sample every 24 h interval (Wuana *et al.*, 2011).

RESULTS & DISCUSSION

Out of several samples only the isolated bacterial strains depicted in Table 2 were found capable for high reduction of toxic chromium (VI) and lead for which we have selected. Further bioremediation study for chromium (VI) and Lead Toxicity using *Bacillus subtilis* and *Bacillus amyloliquifaciens* microbes has been done by using selected bacterial strains.

The various morphological characteristics of efficient reducing strains of Chromium and Lead are summarized in Table 3.

Table 3: Morphological characterization of CRS and LRS

Sr.No	**Morphological characters**	CRS-W	CRS-Yl	LRS-Y2	LRS-R
1	Colony Shape	Circular	Circular	circular	circular
2	Colony color	white	Pale yellow	yellow	Reddish
3	Colony elevation	Convex	convex	convex	convex
4	Colony margin	Rod	cocci	rods	cocci
5	Gram character	+ve	+ve	-ve	+ve

The results obtained by the current study was strongly justified by another similar study by (Srisuwan 2002), which concluded that cells are just not the physical targets in which irradiations instantly bring about mutations, rather, it is how a cell responds to radiations, that determines whether it will result in mutation. Post irradiation treatment have a marked effect on lethality and on yield of mutations.

CONCLUSION

Sustainable development necessitates the advancement and advancement of management of the environment, as well as a continuous exploration for green technologies to treat a wide range of marine and terrestrial habitats that have become polluted as a result of growing anthropogenic activities (Ullah *et.al.*, 2018). BR is becoming a more famous substitute to traditional techniques for treating waste compounds and media, as it allows contaminants to be degraded through natural microbial activity mediated by various microbial strain consortia (Yan *et.al.*, 2019). Furthermore, chromate reducing bacteria (CRS) and lead reducing bacteria (LRS) were isolated for bioremediation studies of chromium (VI) from effluent. As a result of these isolated bacterial strains, only a few bacterial strains capable of high reduction of toxic chromium (VI) and lead have been chosen. Selected bacterial strains were used in a bioremediation study for chromium (VI) and lead toxicity using *Bacillus subtilis* and *Bacillus amyloliquifaciens* microbes.

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