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Chromium Reduction by Chromium Resistance Bacterial Sp Isolated from Tannery Effluents

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Abstract: In modern biotechnology, bioremediation is most become developing field in environmental remediation technology. In this study, find the microorganisms have capable hexavalent chromium to trivalent chromium reduction. The bacterial culture was isolated from the contaminated sites of tannery industries effluents from Tamilnadu, India. The chromium contaminated soil estimated by amount chromium presented in the tannery effluent, the sample number III presented maximum level of chromium (0.480 mg/g) with pH: 8.3 and four types of bacterial cultures were isolated from this tannery effluents ability to reducing hexavalent chromium [Cr (VI)] to trivalent chromium [Cr (III)]. It could chromium tolerate S1, S3, S4, S6 and S8 *Bacillus* sp up to a 1000µg/ml and other microbial sp S2,S5 and S7 tolerate up to 750µg/ml, with the temperature 37°C and pH:7.0 were maintained. The present study was shows most of the *Bacillus* species can attempt to evaluate the status of chromium resistant ability to have in tannery effluent sediments of Tamil nadu based industries.

Key words: Bacterial sp · Chromium · Chromium reduction · Tannery effluent

INTRODUCTION

Chromium is widespread uses in steel production, wood preservation, leather tanning, metal corrosion inhibition, paint and pigments and other industrial applications, the industrial effluent containing hexavalent chromium occurs prilimarily in natural as trivalent or hexavalent form are released directly or indirectly into the natural water resource[1]. Cr (VI) compounds are highly water soluble, toxic and carcinogenic in mammals in contrast, trivalent chromium is considered to be nontoxic as it precipitates at pH higher than 5.5 with the formation of insoluble oxides and hydroxides in soil and water systems. Hexavalent chromium is toxic and mutagenic to most organisms and is known to cause irritation, corrosion of the skin and respiratory track; it also causes lung carcinoma in humans [2, 3].

Biological approach could be initiated by the microbial reduction of Cr (VI). Microbial populations in Cr (VI) polluted environments adapt to toxic concentrations of Cr (VI) and become chromium (VI) resistant and chromium (VI) reducing strains under aerobic and anaerobic conditions [4-6]. In previous researches, a number of microorganisms have been reported to be able to reduce Cr (VI), including strains of, *Bacillus* [7], *Pseudomonas* [8, 9], *Enterobacter* [10, 11], *Shewanella* [12,13] *Escherichia coli* [14] and several other bacterial isolates [15, 16].

The objective of the study, deals with the isolation of hexavalent chromium reducing bacteria from contaminated environment, ability of the bacterium to reduce hexavalent chromium reduction and to localize the chromium reducing activity. These results obtained in this study may provide the useful knowledge for the bioremediation of chromate pollution.

MATERIALS AND METHODS

Sample Collection: Soil sample were collected from three different tannery effluent sediments from Tamilnadu, India. Tamilnadu which was heavily contaminated with chromium industrial wastes including textile processing, dye stuff manufacturing and chrome plating industrial waste. An amount of 150 g of soil from each site is taken, mixed well and dried in the oven at 60°C for 24 h, crushed the sample using a crusher to remove the boulders. A representative sample of the soil was characterized in the laboratory as per standard procedure [17]. The soil characteristics thus determined are given in Table 1.

Estimation of Chromium from Soil Sample: For the estimation of total chromium from soil, an alkaline digestion method and nitric acid/sulfuric acid digestion methods [18] were used, respectively. The hexavalent chromium was measured colorimetrically at 540 nm by

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Table 1: Physical and Microbiological characteristics of tannery waste sediments

Samples	pH	Total chromium mg/g	Microorganismes CFU x 10^6 /g PYE + Cr (100µg ml^{-1})
1	8.1	0.324	1.85
2	7.9	0.228	1.09
3	8.3	0.480	2.79

I. Sempattu Tannery effluent sample,
II. Vaniyambadi Tannery effluent sample.
III. Trichy Tannery effluent sample,

reaction with diphenyl carbazide (DPC) in acidic conditions. In the case of Cr (III), potassium permanganate was used to oxidize Cr (III) to Cr (VI).

Isolation of Chromium Reducing Bacteria: For isolation of Chromium resistance bacteria from tannery effluent sample was serially diluted using distilled water and inoculated in Peptone yeast extract(PYE) Agar plates [19] containing Chromium concentration about 100 µg/ml. The isolates were tested for their resistance to Chromate by growth in PYE agar plates containing various concentrations of Cr^{6+} (100, 250, 500, 750 and 1000 µgs/mL). Which could grow on plate containing chromium was further studied for its reduction of hexavalent chromium to trivalent chromium in liquid medium [19].

Effect of Various Chromium Concentration and Chromium Reduction in Liquid Medium: PYE broth amended with various initial concentration of chromium ranging from 100µg to 1000µg (potassium chromate) was inoculated with overnight grown bacterial culture to obtain the inoculated flasks were incubated at 37°C for shaking at 150rpm. Aliquots (3 ml) were withdrawn at regular time interval and analyzed for growth and chromium reduction. All the experiments were done in triplicates [19].

Biochemical Identification: Various biochemical tests (Indole Production, Methyl Red, Voges -Proskauer, Citrate Utilization, Urease, Catalase, Triple Sugar Iron, Gelatin Hydrolysis, Starch Hydrolysis and Carbohydrate Fermentation etc) were done by bacterial species identification.

RESULT AND DISCUSSION

Estimation of Chromium from Tannery Effluent: The total amount chromium present in the tannery effluent samples were estimated by diphenyl carbozide (DPC) method and pH of the sample also estimated. The values of these parameters were shown in the Table 1. The total Cr content of the contaminated sediments varied from 0.23 to 0.48mg/g. The pH range between 7.0 to 8.3. Megharaj *et al.,* [20] studied in his work; the soil from the tannery waste disposal site used for isolation of Cr (VI)-reducing bacteria contained 40 mg Cr (VI)/ kg in soil.

The wide variety of microbial population was seen in PYE agar plate containing 100µg/ml concentration of Chromium. With in the population only morphologically identified bacterial strains isolated from the plate and purified for further studies [21]. Isolated the bacterial strains have the bacterial counts of 4.2 x 10^6 and 5.0 x 10^5 colony forming units (cfu)/ml were obtained at a chromate concentration of 100 µg/ml. The Similar declines in bacterial populations (cfu) of the Cr-contaminated sediments were also reported by Luli GW; Losi and Frankenberger [21, 22].

Soil sample were collected from three different tannery effluent sediments from Tamilnadu, India. Tamilnadu which was heavily contaminated with chromium industrial wastes including textile processing, dye stuff manufacturing and chrome plating industrial waste. A total of eight bacterial isolates representing the entire different bacterial colony were able to grown on chromium containing plates, Microbial communities responds rapidly to environmental changes and this should be reflected in specific parameters of microbial structure and function. The response of the microbial community to environmental stress is the result of a combination of factors. The bacterium which could tolerate chromium was used for further studies.

Biochemical Identification: Table 2 shows various biochemical characteristics of the strains. Based Reduction of chromium by bacterial isolate on the biochemical analysis the bacterial sp has been identified.

Bacterial Strain Identification

Bacterial strains	Name of the strains
S1	*Bacillus* sp.
S2	*Pseudomonas* sp.
S3	*Bacillus* sp.
S4	*Bacillus* sp.
S5	*Alcaligenes* sp.
S6	*Bacillus* sp.
S7	*Staphylococcus* sp.
S8	*Bacillus* sp.

Above the mention all of them aerobic bacteria have capable to reduce chromium (VI). All *Bacillus* sp. that isolated reduced the chromium (above 50%) effectively and could grow in high concentration of chromium

Table 2: Minimum Inhibitory Concentration

Strain	Cr(VI) concentration 100µg/ml	Cr(VI) concentration 250 µg/ml	Cr(VI) concentration 500 µg/ml	Cr(VI) concentration 750µg/ml	Cr(VI) concentration 1mg/ml	Cr(VI) concentration 1.25mg/ml
S_1	0.802	0.627	0.323	0.165	0.042	-
S_2	0.854	0.601	0.349	0.153	-	-
S_3	0.824	0.645	0.352	0.182	0.063	-
S_4	0.875	0.653	0.367	0.173	0.055	-
S_5	0.847	0.639	0.317	0.149	-	-
S_6	0.911	0.597	0.392	0.164	0.067	-
S_7	0.792	0.591	0.306	0.129	-	-
S_8	0.906	0.672	0.435	0.287	0.092	-

Table: OD value of the 24h grown culture at 540nm in different concentration of Cr (VI).

(= 1mg/ml) in the field. Gregory *et al*., [23] isolated a variety of bacterial strains like Pseudomonads, Flavobacteria, *Enterobacteria* and *Bacillus* sp. From chromium contaminated river sediments. isolated *Bacillus cereus, Bacillus thuringiensis* and *Bacillus sp.* that reduced the chromium (VI) to chromium (III).

Effect of Various Chromium Concentration and Chromium Reduction in Liquid Medium: Seven morphologically distinct Cr (VI) resistant bacterial strains were screened for their Cr (VI) tolerance limit. The isolated strains were introduced to find the minimum inhibitory concentration (MIC) value of chromium. All strains were grown well in 100g/ml chromium concentration. The growth of the individual isolate was decreased with the increasing of chromium concentration. The growth of the individual isolate is decreased in exceeding concentration like 250µg/ml, 500µg/ml, 750µg/ml, 1000µg/ml and 1250µg/ml. With in the eight strains, S_2, S_5, S_7 strains have the Minimum Inhibitory Concentration (MIC) at 1000µg/ml. The strains S_1, S_3, S_4, S_6, S_8 were grown even above 1000µg/ml concentration of Cr (VI). They have MIC value at 1250µg/ml Cr (VI) concentration. In this concentration microbial growth has completely less.

Similar study was reported by Srinath *et al.*, [24], he reported that *Bacillus sp.* has grown upto 1500µg/ml concentration. Chromium resistant bacteria isolated from effluent of tanneries could resist up to 250 µg/ml of Cr (VI) in the medium [19]. Megharaj *et al*, [20] also reported strains, which were isolated from polluted soil, could resist up to 100 µg/ml of Cr (VI). Table 2 represent that the comparison of growth of eight isolated strains in different concentration of chromium and their Minimum Inhibitory Concentration (MIC) value. Similar trends of growth have been reported for *A. oxydans*, which showed tolerance up to 50 µg chromate per ml [20].

Table 3 Bioreduction of Bacterial Strains

Strain No	Amount of chromium (VI) reduced* µg/ml	Percentage of chromium reduction (%)
S_1	44.33	44
S_2	26.65	27
S_3	50.23	50
S_4	44.33	44
S_5	32.97	32
S_6	56.41	56
S_7	18.91	19
S_8	56.41	56

*calculated from the 24 h incubated broth culture containing 100µg/ml of initial Cr (VI) concentration.

Chromium (VI) bacterial resistance above 2500 mg/L has only been reported by Shakoori *et al*., [23]. They isolated a gram positive bacterial strain (probably a *Bacillus* species) from a tannery effluent that grew in media containing potassium dichromate up to 80 mg/ml. Most Cr (VI) - resistant microorganisms are tolerant up to 10 to 1500 mg/L of Cr (VI) [24, 25]. It indicates that the growth of the microbial isolates was depending on the concentration of chromium present in the medium. Present study was six bacterial cultures were isolated from this tannery effluents ability to reducing hexavalent chromium [Cr (VI)] to trivalent chromium [Cr (III)]. It could chromium tolerate S1, S3, S4, S6 and S8 *Bacillus* sp up to a 1000µg/ml and other microbial sp S2,S5 and S7 tolerate up to 750µg/ml, the temperature 37°C and pH:7.0 were maintained.

Chromate Bioreduction: The isolated bacterial have ability to reduce Cr VI to Cr III. The test was taken for the bioreduction of chromium using these isolates. They could reduce the chromium present in the medium have initial concentration about 100µg/ml at 24h incubation period. The percentage of chromium reduction of each isolates was represented in the Table 3.

Table 5 Characterization and Identification of Bacterial Strains

Biochemical tests	S1	S2	S3	S4	S5	S6	S7	S8
Colony morphology	White colony	White Dotted colony	Dispersed white colony	white Solid bed	Small Yellow colony	Small White colony	Small translucent colony	Small White colony
Cell shape	Chain rod	rod	rod	rod	rod	rod	Cocci	rod
motility	motile	motile	motile	motile	motile	motile	Non motile	motile
Gram staining	+	-	+	+	-	+	+	+
Spore staining	+	-	+	+	-	+	-	+
Indole test	-	-	-	-	-	-	-	-
Methyl red	+	-	+	-	+	+	-	+
Vogus proskauer	-	-	-	+	-	-	-	-
Citrate utilization	-	+	-	+	-	-	-	-
catalase	-	+	-	+	+	+	+	+
urease	-	-	-	-	-	-	-	-
TSI	K/A	A/A	K/A	K/A	A/A	A/A	K/K	K/K
Starch utilization	+	+	+	+	-	+	-	+
Gelatine hydrolysis	+	+	+	+	+	+	-	+
Glucose fermentation	-	-	-	-	-	-	-	-
Sucrose fermentation	-	-	-	-	-	-	-	-

The maximum reduction was identified in two S_6 and S_8 isolates that have 56% of reduction of 100µg/ml initial concentration of chromium. The reduction range between 19%-56% was observed in the isolated strains. The reduction rate is depends on the time course that for the incubation period. The reduction rate is increased with the time of incubation. This similar study was taken by Guojun cheng *et al* [26] using *Bacillus sp* and Arthrobacter *sp* and Cr(VI) reduction and bacterial growth with Cr(VI) concentration of 1- 100 mg/L of Cr (VI) reduction rate were rapidly decreased by 100 mg/L of Cr(VI) [27]. The toxic and mutagenic effects of chromium on bacteria have been reported to occur at concentration of 10-12 mg/L Cr (VI), with significantly affected cell growth of *Bacillus* sp. [27]. The toxic effects are altered the genetic materials and metabolic reactions [28].

CONCLUSION

The present study focuses on the ability of a mixed population of microbial culture isolated from hexavalent chromium contaminated site to biotransform Cr (VI). Among the bacterial strains isolated from three different locations of the contaminated site, the bacterial strain (S8) which was showed high Cr (VI) reduction activity. Optimum concentration of bacteria for the reduction of Cr (VI) to Cr (III) was observed as 1000µg/ml, 24h and 37°C respectively. From the above results, it is evident that the tannery waste sediments provide an enriched Cr resistant bacterium could be isolated. In modern biotechnology possible to genetic and enzymatic improvement will be increased the Cr resistance activity.

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