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Characterisation of Dominant Chromate Reducing Bacteria in Soils Surrounding Tannery Industries

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***Abstract*— Objective: To investigate the dominant bacterial isolates in the soil surrounding the tannery industries capable of chromate reduction. Methods: Plate count method to isolate bacteria from soil. Biochemical charcterisation of the bacteria by routine identification methods by bergy's method and determining their chromate reduction potency by dilution plating technique. Results: Four dominant bacterial isolates were identified from the collected soil samples. .Biochemical identification protocol infers the four isolates to be Pseudomonas, Bacillus, Enterobacter and Kelbsiella. The highest concentration of Cr(VI) was reported as 250µg/ml.**

***Index Terms*— Bacteria; Tannery industry soil; biochemical charetristion; Cr((VI) reducing potency.**

I. Introduction

To stop pollution and to prevent metal-toxicity there is a clear need for an overall waste treatment strategy with the goal of elimination of priority pollutants at source. This can be achieved by indigenous microorganisms found in various industrial effluents which can be used as an indicator of pollution and can be used to resist, process, metabolize and detoxify chromate polluted waste water (1). Biological reduction of hexavalent chromium using indigenous micro-organisms offers a new cost effective and environmentally compatible technology. Early investigations demonstrated that facultative anaerobic bacteria such as *Pseudomonas dechromaticans* (2), *Pseudomonas chromatophila* and *Aeromona sd echromatica* remove hexavalent chromium from solution by the formation of a hexavalent chromium precipitate, presumably $Cr(OH)_3$. Subsequent studies have shown that the capacity for hexavalent chromium reduction is widespread and is reported in organisms such as *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Achromobacter eurydice, Micrococcus roseus and Escherichia coli.*

II. Biochemical Identification

The isolates found predominant in tannery industry soil were inoculated in peptone water and incubated overnight. Fresh broth culture of these isolates were inoculated aseptically on nutrient agar plates and assigned with diverse concentration of chromium as from 25-500µg/L. The prevalent isolates grown in all the concentrations were selected and pooled out. It was then validated as the final concentration and assigned as 250µg/L (showed maximum growth). Morphological and cultural characteristics such as abundance of growth, pigmentation, optical characteristics, form, size, margin and elevation were studied on nutrient agar plates. Presumptive genus level identification was done by employing routine morphological and biochemical identification tests. For further identification, characterization and confirmation, biochemical tests were employed in the accordance to the Bergey's manual of Determinative bacteriology (Holt et al., 2006).

III. RESULTS

The isolates were spotted based on their colony morphology. Among the inhabitant isolates, four dominant ones were selected for the study based the tolerance ability towards various concentration of Cr (VI) source as described in table 1.The above mentioned isolates were characterized through the morphological and biochemical procedures. These isolates when inoculated in varying concentrations of Cr(VI) were able to multiply. But the growth pattern differed with the concentrations. It is inferred from the table 1 that increasing concentration of Cr(VI) did not affect the growth drastically. But the number of CFU started to decrease after 250 µg/ml. Thus 250 µg/ml was selected as the standard concentration of Cr(VI).

As per the morphological and biochemical characterization (Table 2) the bacteria were identified and further they were confirmed by selective plating. *Pseudomonas* was plated *Pseudomonas* isolation agar, *Bacillus* in Mannitol Salt Agar, *Klebsiella* in MacConkey agar and *Enterobacter* in *Enterobacter* isolation agar. The isolates were belonged to the genus namely *Pseudomonas sp* , *Bacillus sp* , *Enterobacter sp* and *Klebsiellasp.*

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Table1; Prevalence of chromate tolerant isolates in the samples

S.No	Concentration of Cr(VI) (µg/ml)	C.F.U/ml of samples) (average of three replicates)
1	25	82
2	50	75
3	75	62
4	100	48
5	125	32
6	150	24
7	175	22
8	200	20
9	225	18
10	250	15
11	300	12
12	350	10
13	400	9
14	450	5
15	500	4

Table 2 Biochemical characteristic of chromium tolerance isolates isolated from the soil samples

S.No	Test	*Pseudomonas*	*Bacillus*	*Enterobacter*	*Klebsiella*
1	Gram reaction	Gram-Negative rods	Gram-Positive rods	Gram-Negative rods	Gram-Negative rods
2	Motility	Motile	Non-Motile	Motile	Non-Motile
3	Catalase	Positive	Positive	Negative	Positive
4	Oxidase	Positive	Positive	Negative	Negative
5	Indole production	Negative	Negative	Negative	Negative
6	Methyl red	Negative	Negative	Positive	Negative
7	Voges-Prosakauer	Negative	Positive	Positive	Positive
8	Citrate	Positive	Negative	Positive	Positive
9	Urease	Positive	Negative	Negative	Positive
10	Starch hydrolysis	Negative	Positive	Negative	Positive
11	Nitrate reduction	Positive	Positive	Positive	Positive

DISCUSSION

Many microorganisms have been found to tolerate an extremely high concentration of heavy metal ions and relieve the toxicity. Thus the advantage of these characteristics and mechanism of the resistance of heavy metals of some microorganisms are used in the hazard-free treatment of various heavy metal polluted environments (3). These bacterial isolates were native and thus being able to tolerate different amounts of chromium in the growth media (4). This capacity was employed to indentify the maximum concentration of Cr(VI) that could be added in the growth media. This also infers the Cr(VI) reducing property of the bacterial isolates (5). When inoculated in varying concentrations of Cr(VI) the bacterial isolates were able to multiply exhibiting a diversified growth morphology and count. When concentrations were increased its found that the count of the bacterial isolates maintained almost the same. Thus the maximum tolerance limit was selected. These isolates could be employed for biological treatment of Cr(VI) pollution(6). Morphological and biochemical analysis of the dominant chromium tolerant bacterial isolates were done to reveal their identity. They were *Pseudomonas(7) Bacillus* , *Enterobacte(8)* and *Klebsiella.*

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