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Plasmid Mediated Chromium Degradation by Bacteria

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Microorganisms are used in a number of ways to decontaminate polluted areas and stimulate the environment. The extensive industrial usage of chromium compound & subsequent release of effluent in the environment contaminates the ecosystem. Effluents or discharges from these industries have been found to contain high concentration of this metal. The isolation of chromium resistant bacteria and evaluation of their chromium detoxification are primary steps in developing bioremediation processes. Chromate resistance in bacteria is either chromosomal or plasmid determined. Plasmid borne chromate resistance has been found in many bacteria. In the present study chromate resistant bacteria is isolated from the tannery sites. The chromate reducing capacity of the isolates is studied along with their plasmid profile. Plasmid curing is done in all the isolates and their chromate reduction capacity is reviewed. We do conclude that chromate resistance in bacteria is plasmid mediated in the selected bacterial species.

Key words: Chromium, Plasmid, Bacteria and Curing.

Chromium is a heavy metal that has many user in the metallurgic, refractory, chemical and tanning industries. While chromium (VI) complexes were one of the first classes of chemicals to be implicated as human carcinogens. Chromium (III) has generally been considered as one of the least toxic of the transition metals (Nrigan and Nietoer 1988). Chromium is a classified group A carcinogen based on its chronic and subchronic effects (Anderson, 1988).

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Hexavalent chromium has many industrial application and often causes environmental contamination in marine and freshwater sediments from urban and industrial discharges (Losi *et al.,* 1994). Chromium contamination is common in soil, ground and surface waters in industrial areas. Chromium compounds have been released. Every regulatory agency has listed chromium (Cr^{6+}) as a priority toxic chemical for control, with the maximum allowable level in drinking water of 50-100μgL^{-1} (Tchobanoglous and Burton, 1991). A broad variety of bacteria reduce chromates. Bacterial resistance to Ag+, ASo(-2), ASo-4(3-), Cd(2+), Co(2+), Cro4(2-), Hg+ are plasmid encoded. Several copper and chromate resistance bacterial strains were isolated from the effluent of leather tanning industries (Guibal *et al.,* 1999). *Bacillus strain* QCI 2 isolated from chromium polluted zone was selected by its high ability to both tolerate and reduce hexavalent chromium to less toxic trivalent chromium (Compos, 1998). Recently, heavy metal accumulating strains has sought a new role as small factories for production

of "nano particles" (Joerger *et al.,* 2001). Camargo *et al.,* 2003 have studied the chromium reduction by bacterial consortium isolated from soils contaminated with dichromates.Chromate resistance of *pseudomonas fluorescens* LB 300, isolated from chromium contaminated sediment in the upper Hudson river, was found to be plasmid specified – loss of plasmid by spontaneous segregation or mitomycin C curing resulted in a simultaneous loss of chromate resistance (LH Bopp. 1987). Cervantes and Silver (1992) reported that chromate pollution has promoted the selection of bacterial strains possessing chromate resistance determinants, usually carried by plasmids. Strains combining both abilities that is resistance and reduction of chromate are potentially useful for detoxifying chromate polluted wastewater. Ohtake *et al.* (1990) reported that chromate resistance in *Pseudomonas fluorescence* LB 300 was reported to have reduce uptake of chromate relative to the plasmid less strain chromate transport by resistant cells. The genes for a hydrophobic polypeptide, Chr A, were identified in chromate resistance plasmids of *Pseudomonas auroginosa* and *Alcaligens eutrophus* (Cervantes and Silver 1992). Hence the present study focuses on the plasmid borne chromate resistance and and the influence of curing on resistance which is an prerequisite for chromate degradation.

MATERIAL AND METHODS

Collection of wastes

Soil and effluent samples were collected from a tannery industrial site near Begampur, Dindugal district in Tamil nadu. The samples were collected in sterile containers and transported to the laboratory and stored at 4^0C **.**99g or 9ml of sample was taken and serially diluted. Three dilutions (10^{-3},10^{-4},10^{-5}) were selected and plated by spread plate technique using nutrient agar medium. Single isolated colonies were selected for further study.Predominant isolates were found among the isolated colonies. Among the isolates found four district types were found to be predominant in all dilutions. They were selected for further studies.

Identification of the isolates

The dominant isolates were identified according to the standard procedures from simple staining to IMVIC tests followed by their confirmatory test like growth in the specific media. Thereby four different dominant isolates were selected for further steps in the experiment.

Chromium uptake studies

Different concentration of chromium were prepared by using potassium dichromate as a source of chromium. Four dominant isolate were inoculated in the solutions separately. This setup was maintained for 72hours. Samples were withdrawn for every 24hours interval and analysed for total viable count and residual chromium.

Analysis of residual chromium

Residual chromium were analysed by 1,5- Diphenyl carbazaide assay. 1ml of culture broth was centrifuged at 10,000 rpm for 10 min and 0.5 ml of supernatant was taken, and 1ml of 3M sulphuric acid was added to get a concentration of acid 0.1M. 0.5ml of 1,5 – Diphenyl carbazide (0.25% in 50% autone) was added to solution to make up the volume 10ml. Then the residual chromium was determined spectrophotometrically at 540nm.

Plasmid curing

Tubes containing 25ml of peptone water was supplemented with acridine orange (20µg/ m1), and inoculated with 0.1ml of overnight culture. Then it was incubated at 37°C for 24hrs. There are other methods like high temperature (45°C), sodium dodecyl sulphate (0.1 to 2%) were used to cure chromate resistant plasmids from the chromate resistant strains. A loopful of bacterial growth was resuspended with 100ml of SDS (4.5%) and incubated at 45°C for 15 minutes. After 15 minutes, the contents were plated onto simple nutrient agar plates. The bacterial colonies appearing after 24h of incubation were screened for chromate sensitivity and plasmid loss. The consequent loss of chromate resistance phenotype with the loss of plasmid in all the bacterial strains confirmed that chromate resistance in these strains is conferred by plasmids.After curing of plasmids, the content of chromium was determined by the same procedure as mentioned earlier.

Isolation of plasmid dna (before and after curing)

Plasmid DNA was isolated by alkaline lysis method and then separated in agarose gel electrophoresis using 1% agarose gel.

RESULTS AND DISCUSSION

The selected four isolates were identified by the routine morphological and biochemical tests and the results are represented in table.1

Chromium Reduction Experiment

When the chromium solution, was inoculated with *Bacillus* and incubated for 72 hours, the concentration of chromium was reduced from the initial amount, it reduces 50 µg/ml of chromium to 16mg after 72 hours where as *Bacillus* reduces 250 µg/ml to 65 µg/ml after 72 hours. When *Bacillus* was cured and then subjected to treatment 50µg/ml was reduced to 34 µg/ml and 200 µg/ml was reduced to 70µg/ml. Thus the performance of the strain were better before curing. (Table 2). When 250 µg was supplied as an initial concentration *Pseudomonas sp* was able to reduce it to 45µg/ml after 72 hours. About 12µg/ml was got when 100µg/ml was supplied. The cured strains where able to reduce 200µg/ml to 57 µg/ml and 250µg/ml to 80µg/ml. (Table 3). *Enterobacter sp* exhibited the poorest performance in Cr (VI) reduction. When 50µg/ml was supplied it was reduced to 24µg/ml. 150µg/ml was reduced to 98 µg/ml. After curing the reduction was still megare. 250µg/ml was reduced to 196 µg/ml and 50 µg/ml to 31µg/ml. (Table 4). The last organism employed was *Klebsiella sp*. This exhibited better Cr(VI) reduction capacity. When 100µg/ml was supplied it was able to reduce it to 66µg/ml. 150 µg/ml was reduced to 102 µg/ml. After curing the reduction was still reduced. 250 µmg/ml was reduced to 175 µg/ml and 100 µg/ml was reduced to 73 µg/ml (Table 5).

Among the four organisms employed the performance of *Bacillus sp*. was next to *Pseudomonas sp*. But the reduction was higher than *Klebsiella sp*. and *Enterobacter sp*. Thus these results implicate that the Bacillus sp. isolated from chromium polluted zone has better reduction ability. Similar reports were given by Compos *et al*. (1995). Plasmids were isolated and

Table 1. Characteristics of *Bacillus* sp*, Pseudomonas* sp, *Enterobacter* sp and *Klebsiella sp*

Test	*Bacillus*	*Pseudomonas*	*Enterobacter*	*Klebsiella*
Gram reaction	Gram-Positive	Gram-Negative rods	Gram-Negative rods	Gram-Negative rods
Motility	Non-Motile	Motile	Motile	Non-Motile
Catalase	Positive	Positive	Negative	Positive
Oxidase	Positive	Positive	Negative	Negative
Indole production	Negative	Negative	Negative	Negative
Methyl red	Negative	Negative	Positive	Negative
Voges-Prosakauer	Positive	Negative	Positive	Positive
Citrate	Negative	Positive	Positive	Positive
Urease	Negative	Positive	Negative	Positive
Starch hydrolysis	Positive	Negative	Negative	Positive
Nitrate reduction	Negative	Positive	Positive	Positive

Table 2. Reduction of chromium by *Bacillus sp*

S.	Chromium	Before curing µg/ml			After curing µg/ml		
No.	Concentration µg/ml	24	48	72	24	48	72
1.	50	38	29	16	48	42	34
2.	100	62	30	26	93	84	79
3.	150	98	45	23	132	116	82
4.	200	153	76	31	180	94	70
5.	250	216	124	65	238	182	38

Table 3. Reduction of chromium by *Pseudomonas* sp

S. No.	Chromium Concentration µg/ml	Before curing µg/ml			After curing µg/ml		
		24	48	72	24	48	72
1.	50	34	26	15	42	35	24
2.	100	72	32	12	84	51	38
3.	150	124	63	28	139	96	65
4.	200	146	76	20	163	105	57
5.	250	210	132	45	236	172	80

Table 4. Reduction of chromium by *Enterobacter* sp

S. No.	Chromium Concentration µg/ml	Before curing µg/ml			After curing µg/ml		
		24	48	72	24	48	72
1.	50	46	35	24	48	39	31
2.	100	92	84	60	96	90	81
3.	150	137	112	98	143	130	109
4.	200	174	162	139	188	169	159
5.	250	224	216	185	239	228	196

Table 5. Reduction of chromium by *Klebsiella sp*

S. No.	Chromium Concentration µg/ml	Before curing µg/ml			After curing µg/ml		
		24	48	72	24	48	72
1.	50	33	27	21	41	35	29
2.	100	85	78	66	93	89	73
3.	150	130	119	102	141	125	113
4.	200	163	125	97	181	149	104
5.	250	218	186	153	242	224	175

separated by agarose gel electrophoresis revealed the presence of plasmids in all the four isolates. After curing plasmid were lost in two organisms namely, *Enterobacter* and *Klebsiella* (Sultan and Hasnain 2005). When the cured cultures were used in the chromate reduction experiment. The performance of the cultures after curing were very less when compared to their performance before curing. Similar reports were given by Verma *et al.,* (2004).Chromium reduction was also seen after curing to a lower extent suggesting that chromate resistance. In bacterial strains is determined by both plasmid and chromosome (Cervantes and Silver 1992). The same reports were found in this study.

Plasmid genetic determinants for chromate resistance have been described in several bacterial genera, most notably in *Pseudomonas*. Chr A genes were identified in chromate resistance plasmids of *P. aeruginosa* and *Alcaligens eutrophus* (Cervantes and Silver, 1992). Hence curing of plasmids were carried out this study. Hence the results of this study throw light on the influence of plasmid in chromate resistance and futher genetic manipulation studies in this area will yield fruitful results in developing a novel organism to clean up chromate polluted environments.

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