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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (3): 450-456, 2000

Hexavalent Chromium Resistant Bacteria from Effluents of Electroplating: Isolation, Characterization and Chromium (VI) Reduction Potential

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Abstract: Ten hexavalent chromium resistant bacterial strains SECr-1, SECr-2, SECr-3, SECr-4, SECr-5, SECr-6, SECr-7, SECr-8, SECr-9 and SECr-10 were isolated horn the effluents of three electroplating units situated in Gujranwala. These bacteria exhibited a very high level of resistance to hexavalent Cr salt and could bear more than 40 mg ml^{-1} of potassium chromate in nutrient agar medium. All of them had yellowish white (except SECr-7 which had yellowish brown, SECr-6 and SECr-8 which had off white), convex and circular colonies with entire margins. The cells of these strains were G-ve, motile, aerobic rods. Their morphological and biochemical attributes align them with family Pseudomonadaceae. The optimum temperature for the growth of these bacterial strains was 37°C both in the absence and presence of chromate except SECr-7 fin the absence), SECr-1, SECr-2 and SECr-5 (in the presence of chromate) which yielded maximum growth at 28°C. All of them were capable of growing in wide pH range (pH 5 to pH 9) with maximum growth at pH 7 or 8. However, in the presence of chromate in the medium they preferred alkaline pHs (pH 8 or 9). These bacterial. strains also conferred resistance against salts of other metals and antibiotics. These bacterial strains had great potential for hexavalent chromium reduction and can be exploited for hexavalent chromium detoxification.

Key words: Cr (VI) resistant bacteria, electroplating effluents, Cr (VI) reduction, bioremediation

Introduction

Chromium is discharged into the environment through large number of industrial processes including electroplating, metal refinishing, leather tanning, inorganic chemicals production, etc., (Wang and Xiao, 1995) and thus is a major environmental contaminant (Cheng *et al*., 1998; Zhitkovich *et al.,* 1998). United States EPA has estimated the Cr discharges from these industrial processes at approx. 10,000 lb per day (Towil *et al*., 1978) and Cr concentration as high as 2740 mg/l has been found in ground water (Office of Technology Assessment, 1984). Electroplating along with leather tanning and textile industries are considered to be the major metallurgical waste water producing industries of Pakistan. There are about 1150 electroplating units throughout the country which discharge upto 23000 m^3 of highly toxic waste water every day. A few composite samples of plating wastes in Pakistan have been shown to contain 0.62-4.8 mg l^{-1} of Cr (VI) along with other metals (Rahman and Stall, 1996). Inspite of its crucial role in biological life, above critical level, it has diverse cellular and molecular effects. The toxic, mutagenic, carcinogenic, genotoxic and teratogenic effects of Cr are well characterized (Sugiyama, 1994; Asmatullah and Shakoori, 1998; Cheng *et al*., 1998; Hartwig, 1998; Singh *et al*., 1998; Markovich and James, 1999). In addition it is common allergen (Lansdown, 1995; Proctor *et al*., 1998) and cause DNA damage (Bose *et al*., 1998). Once inside the cell Cr^{6+} is reduced to Cr^{3+} via intermediate forms, which stably binds and interact with nucleic acids (Singh *et al*., 1998).

It is, therefore, essential to detoxify/extract the toxic chromium (VI) from effluents before discharging in the environment. The current treatment processes for contaminated chromium generally involves the chemical reduction of Cr^{6+} to Cr^{3+}, ion exchange or electrodepositing. Most of these methods are very expensive. Hence more economical methods are being explored (Ohtake and Silver, 1994; Fujie *et al*., 1996). The use of microorganisms offer an inexpensive alternate for treatment of contaminated sources. Bacterial potential for enzymatic reduction of Cr^{6+} to Cr^{3+} (Fujie *et al*., 1996; Chirwa and Wang, 1997; Turick *et al.,* 1996,1997; Tucker *et al*., 1998) offer an alternative candidate for treatment of toxic Cr (VI). A bioprocess for Cr (VI) reduction has several economical and operational advantages over routine Cr (VI) treatment processes (Ohtake and Silver, 1994; Turick *et al*., 1997). There is evidence for both aerobic (Ishibashi *et al*., 1990; Garbisu *et al*., 1998) and anaerobic (Turick *et al*., 1996) reduction systems with different microbes.

Materials and Methods

Three effluent samples were collected from three electroplating units (Barg Industries- Electroplating, Gujranwala, Tariq Industries -Nickel and Chrome Plating, Sialkot Road, Gujranwala, Anwar Industries, G. T. Road, Gujranwala) in sterile screw capped glass bottles. For the isolation of hexavalent Cr resistant bacteria, aliquots were plated on nutrient agar (Gerhardt *et al*., 1994) supplemented with 100 μg ml^{-1} of potassium chromate and incubated at 37°C. Apparently distinct colonies were picked and purified. The purified hexavalent chromium resistant isolates were then gradually exposed to increasing concentration of potassium chromate in the medium. The isolates which could endure upto 40 mg ml^{-1} of K_2CrO_4 in the medium were characterized morphologically and biochemically ensuing Gerhardt *et al*. (1994). Additional 21 biochemical tests were performed with QTS-20 (Quick Test Strips) and CO (Cytochrome Oxidase) strips (DESTO Laboratories, Karachi, Pakistani. The spore forming ability of the isolates was ascertained by tetrazolium overlay method of Moir (1981).

The effect of varying temperatures (28°C, 37°C, 45°C), pHs (pH 5- pH 9) and pHs in combination with temperatures on the bacterial growth was studied both in the absence and presence of 10 mg ml^{-1} of potassium chromate in the medium. The effect of varying concentrations of potassium chromate was determined in rich medium (0-40 mg ml^{-1} potassium chromate) as well as in M9 minimal medium (0-6 mg ml^{-1} potassium chromate). All the Cr (VI) resistant bacterial strains were also screened for resistance to the salts of other metals ($BaCl_2$, $CdCl_2$, $CoCl_2$, $CuSO_4$, $FeCl_3$, $HgCl_2$, $MnSO_4$ $NiCl_2$, $Pb(NO_3)_2$ and $ZnSO_4$) and different antibiotics (ampicillin, cefradine, cefadroxil, chloramphenicol, cyprofloxacin, doxycillin hyclate, kanamycin, streptomycin, tetracycline).

For the estimation of Cr (VI) reduction potentials of these Cr (VI) resistant strains, the medium used by Deleo and Ehrlich (1994) was

used. The medium containing about 100 μg ml^{-1} of chromate (Cr VI) was inoculated with 1% of inoculum from over night bacterial cultures and incubated at 37°C with shaking (150 rpm). After 24 hours the samples were centrifuged and remaining chromate in the supernatant was determined spectrophotometricafly by diphenylcarbazide method (APHA, 1989).

Results and Discussion

Electroplating are one of the major industries in Pakistan discharging harmful toxic wastes high in metallic contents into the environment. These wastes are dumped untreated either in the open ground or in drains leading to the rivers or seas. This leads to the contamination of the surface and groundwater and thus causing threat to aquatic and terrestrial life (Mir and Hai, 1999). The waste water generated by electroplating is highly toxic in nature because of the presence of toxic metals like Cu, Zn, Ni, Cd and Cr and highly toxic inorganic acids, alkalies and cyanide (Rahman and Stall, 1996). Therefore, it is essential to treat/detoxify toxic Cr in the wastes before discharging into the environment. Bacterial potential for enzymatic reduction of Cr 4V1) to Cr (at) (Wang ald Xiao, 1995; Turick *et al*., 1996) offers an alternative candidate for detoxification of contaminated sources. In this paper the isolation and characterization of Cr (VI) resistant bacteria and assessment of their Cr (VI) detoxification potentials are being discussed. Ten hexavalent chromium resistant strains, SECr-1, SECr-2, SECr-3, SECr-4, SECr-5, SECr-6, SECr-7 (Barg Industries), SECr-8 (Tang Industries), SECr-9 and SECr-10 (Anwar Industries) were isolated from the effluents of electroplating industries which could endure 40 mg ml^{-1} of potassium chromate in the nutrient agar medium. Isolation of Cr resistant bacteria has been reported by many workers (Nair and Krishnamurthi, 1991; Fude *et al*., 1994; Wang and Xiao, 1995). The resistance level of these strains is quite high as compared to the Cr resistant strains isolated by other workers. These Cr (VI) resistant isolates had convex and circular colonies with entire margins. The colonies were yellowish white in color except SECr-7 (yellowish brown), SECr-8 and SECr-10 (off white) with size ranging from 1.0 to 3.0 mm.

All these strains were aerobic, motile gram -ve rods. Other biochemical characteristics of Cr (VI) resistant isolates are shown in Table 1. The morphological and biochemical characteristics of these Cr (VI) resistant bacteria associate them with the family Pseudomonadaceae, especially the genus *Pseudomonas* (Krieg and Holt, 1984). Previously many Cr resistant *Pseudomonas* spp. have been reported (Nair and Krishnamurthi, 1991).

Temperature is an important physical factor which affect microbial cells by influencing the rates of biochemical reactions and enzyme synthesis (Chaloupka, 1985) and at extreme temperatures one or more processes become rate limiting (Patterson and Gillespie, 1972). The optimum temperature for growth of these isolates was 37°C both in absence and presence of chromate (10 mg ml^{-1}) in the medium (Fig. 1a) except SECr-1, SECr-2, SECr-5 (in the presence of chromate) and SECr-7 (in the absence of chromate) which showed best growth at 28°C. The growth was seriously hampered by high temperature especially in the presence of chromate salt. An increased temperature causes changes in membrane composition (Benschoter and Ingram, 1986), imbalance between synthesis and degradation of cellular proteins and ultimately cell death (Strnadova *et al*., 1991). Adverse effects of high temperature appears to have become aggravated in the presence of chromate, pH is another important environmental factor which controls the growth of microorganisms. The Cr (VI) resistant isolates were able to grow over a wide pH range of pH 5 to pH 9, but in the presence of chromate salt (10 mg ml^{-1}) they exhibited poor growth at acidic pH levels. The optimum pH for growth was either 7 (SECr-1, SECr-6) or 8 (SECr-2, SECr-3, SECr-5, SECr-8, SECr-9, SECr-10) while SECr-7 yielded same population density at pH 7 and pH 8 (Fig. 1b). Whereas in chromate supplemented medium the strains generally preferred alkaline pHs (pH 8 or pH 9) for their best growth (Fig. 1b). The strains SECr-2, SECr-3, SEr-5, SECr-6 and SECr-8 opted for pH 8 while the growth of other strains excelled at pH 9. According to Francis (1990) change in pH affect the ionic state of metals that inturn affect the microbial growth. Solubility and availability of metallic salts is more at acidic pH (Hughes and Poole, 1991) which results in poor bacterial growth at acidic pHs. In order to investigate the synergistic effects of pH and temperature on growth of these Cr (VI) resistant isolates, the effect of varying pHs (5-9) in conjunction with varying temperatures (28°C, 37°C, 45°C) was studied. These strains expressed best growth at 37°C with pH 7 (SECr-1, SECr-4, SECr-6) or pH 8 (SECr-2, SECr-3, SECr-5, SECr-8) and at 28°C with pH 8 (SECr-7) or pH 9 (SECr-9, SECr-10) (Fig. 2a). Whereas in chromate supplemented media best growth was exhibited at 37°C with pH 8 (SECT-2, SECr-3, SECr-5, SECr-6) or pH 9 (SECr-1, SECr-4, SECr-7, SECr-9, SECr-1O) but SECr-8 manifested best growth at 28°C with pH 9 (Fig. 2b). In the absence of chromate all the strains were alkaliphilic at 28°C with maximum growth at pH 8 or pH 9 but at 37°C SECr-1, SECr-4 and SECr-6 showed neutrophilic behavior and rest of the strains were alkaliphilic with pH optima of 8. Whilst at 45°C the behavior of these strains was alkaliphilic (SECr-1, SECr-3, SECr-4, SECr-8), neutrophilic (SECT-2, SECr-5, SECr-7, SECr-9, SECr-10) or acidophilic ISECr-6). In the presence of chromate however, all the strains exhibited alkaliphilic behavior at 28°C and 37°C (excluding SECr-7 which was neutrophilic at 28°C) with maximum growth at either pH 8 or pH 9. At 45°C the behavior of the strains was variable. Majority of the strains were alkaliphilic (SECr-1, SECr-2, SECr-3, SECr-8, SECr10) while some strains showed acidophilic behavior (SECr-5, SECr6) or neutrophilic (SECr-4, SECr-7 arid SECr-91 behavior. These , results indicate that pH and temperature had synergistic effects on the growth of Cr (VI) resistant strains. The synergistic effects of pH and temperature on bacterial growth are well established (Sabri *et al*., 1993; Hasnain *et al*., 1993; Hasnain and Abbas, 1997). Fig. 3a shows the influence of varying concentrations of K_2Cr0_4 (0 to 40 mg ml^{-1}) on the growth of Cr (VI) resistant strains in rich medium (nutrient broth). Majority of the strains were able to grow at higher concentration of chromate i.e., 40 mg ml^{-1}. The influence of chromate on the growth of these strains *was* also studied in minimal medium M9. It is obvious from the results (Fig. 3b) that all the strains yielded good growth upto 6 mg ml^{-1} of chromate except SECr-9 and SCEr-10 where growth was drastically reduced atter 3.0 mg ml^{-1} and 2.0 mg ml^{-1} of chromate in the medium respectively. The level of resistance in M9 minimal medium is relatively low as compared to rich medium. In nutrient rich medium cornplexing of the Cr-salt might be lowering the level of available Cr, hence leading to an apparently increased resistance of these strains in rich medium and lower resistance in the minimal medium. In general bacterial population density decreased with increase in salt concentration in the medium. The decreased growth of these strains at higher concentration of chromate might be attributed to increase in generation time, decrease in cell division/cellular multiplication (Al-Aoukaty and Appanna, 1990). Whereas Nair and Krishnamurthi (1991) demonstrated in *P. aetuginose* a decrease in protein, DNA, RNA, sugar and lipid contents, at higher concentration of Cr. The strains were also screened for resistanc to the salts of other metals. All the Cr (VI) resistant strains exhibited resistance to 100 μg ml^{-1} each of BaCI,, $CdCl_2$ (except SECr-3, SECr-6, SECr-8), $CoCl_2$ (except SECr-1, SEC -2, SECr-3, SECr-9, SECr-10), $CuSO_4$,

Table 1: Morphological and biochemical characteristics of hexavalent chromium resistant bacterial strains isolated from electroplating effluents

Sr. No.	Character/Test	Bacterial Strains SECr-									
		1	2	3	4	5	6	7	8	9	10
1	Gram staining	-	-	-	-	-	-	-	-	-	-
2	Shape	rod	rod	rod	rod	rod	rod	rod	rod	rod	rod
3	Motility	+	+	+	+	+	+	+	+	+	+
4	Spore formation	-	-	-	-	-	-	-	-	-	-
5	Cytochrome oxidase	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
6	Catalase	+	+	+ +	+ +	+ +	+	+	+ +	+ +	+ +
7	O. F.	-	-	-	-	-	-	-	-	-	-
8	Methyl red	-	-	-	-	-	-	-	-	-	-
9	Nitrate reduction	-	-	-	-	-	-	-	+	+	+
10	Denitrificetion	-	-	-	-	-	-	-	-	-	-
11	Starch hydrolysis	-	-	-	-	-	-	-	W +	+	+
12	ONPG	W +	W +	W +	W +	W +	W +	W +	W +	W +	W +
13	Sodium citrate	-	-	-	-	-	-	-	-	-	-
14	Sodium malonate	-	-	-	-	-	-	-	-	-	-
15	Lysine decarboxylase	-	-	-	-	-	-	-	-	-	+
16	Arginine dihydrolase	-	-	-	-	-	-	-	-	-	-
17	Ornithine decarboxylase	-	-	-	-	-	-	-	-	-	-
18	11_2S production	-	-	-	-	-	-	-	-	-	-
19	Urea hydrolysis	-	-	-	-	-	-	-	-	-	-
20	Tryptophan deaminase	-	-	-	-	-	-	-	-	-	-
21	Indole	-	-	-	-	-	-	-	-	-	-
22	Acetoin	-	-	-	-	-	-	-	-	-	-
23	Gelatin hydrolysis	-	+	+	+	+	+	+	W +	+	+
24	Acid from glucose	-	-	-	-	-	-	-	-	-	-
25	Acid from maltose	-	-	-	-	-	-	-	-	-	-
28	Acid from sucrose	-	-	-	-	-	-	-	-	-	-
27	Acid from arabinose	-	-	-	-	+	+	+	+	-	W +
28	Acid from rhamnose	+	+	+	+	+	-	-	-	+	+
39	Acid from sorbitol	-	-	-	-	-	-	-	-	-	W +
30	Simon citrate agar	+	+	+	+ +	+	+ +	+ +	+		+
31	MacConkey agar	W +	-	-	-	-	-	+	-	+	+
32	EMB agar	+ +	-	+	-	-	-	-	-	-	-
33	Brilliant green agar	-	-	-	-	-	-	-	-	-	-

+ +, strongly positive; +, positive; W +, weak positive; -, negative

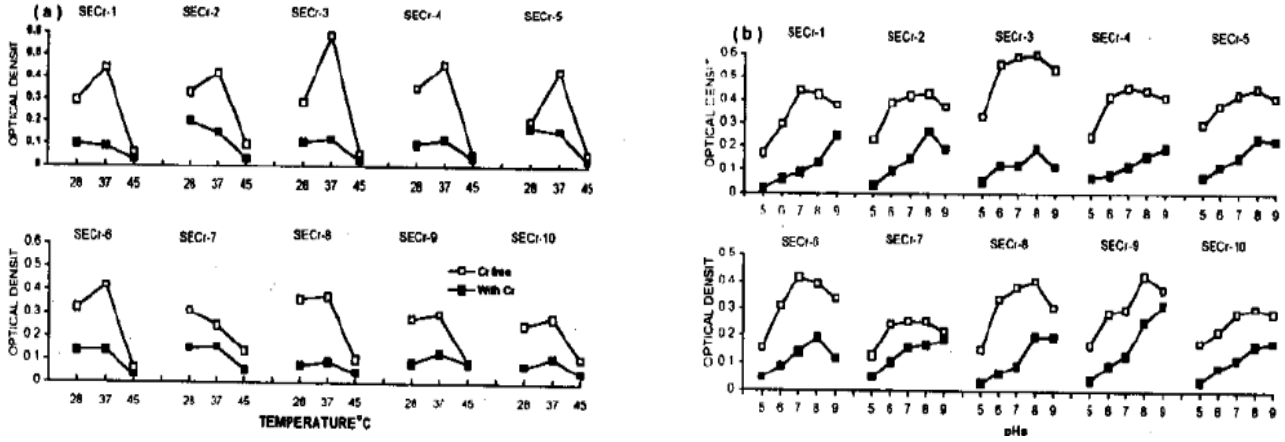

Fig. 1: Growth response of Cr (VI) resistant bacterial strains at varying temperatures (a) and pHs (b) both in the absence and presence of 10 mg ml^{-1} of k_2CrO_4. Bacterial cells were grown in nutrient broth adjusted at desirable pH and incubated at specific temperature for 24 hours. The bacterial growth was monitored at 600 nm

Table 2: Resistance pattern of hexavalent chromium resistant bacterial strains W other metallic salts and various antibiotics. The strains were inoculated on nutrient agar supplemented with desirable concentration of metallic salt or antibiotic and incubated at 37'C for 24 hours

Strains	Metals - 100 μg/ml									
	Eta^{++}	Cd**	Co^{++}	Cu^{++}	Fe^{+++}	Hg^{++}	Mn^{++}	Ni^{++}	Pb**	Zn^{++}
SECr - 1	+ +	W +	-	+ +	+ +	-	+ +	+ +	+ +	+ +
SECr - 2	+ +	+ +	-	+ +	+ +	-	+ +	+ + .	+ +	+ +
SECr - 3	+ +		-	+ +	+ +	-	+ +	+ +	+ +	+ +
SECr - 4	+ +	+ +	+ +	+ +	+ +	-	+ +	+ +	+ +	+ +
SECr - 5	+ +	+	+	+ +	+ +	-	+ +	+ +	+ +	+ i-
SECr - 6	+ +	-	+	+ +	+ +	-	+ +	+ +	+ +	+ +
SgCr - 7	+ +	W^{+}	W^{+}	+ +	+ +	-	+ +	+ +	+ +	+ +
SECr - 8	+ +	-	+	+ +	+ +	-	+ +	+ +	+ +	+ +
SECr - 9	+	+ +	-	+	+	-	+	+ +	+ +	+ +
SECr- 10	+	+ +	-	+	+ +	-	+ +	+ +	+ +	+ +

Strains	Antibiotics - μg/ml								
	Ampicill-in- 300	Cefradin -100	Cefadro-xil -100	Cyprofl-oxacin-100	Chloram-phenicol -5	Doxycil-line- 100	Kanamy-cin - 50	Strepto-mycin-500	Tatracyc-line - 20
SECr -1	+ +	+ +	+ +	-	+ +	+ +	+ +	-	+ +
SECr - 2	+ +	+ +	+ +	-	+ +	+ +	+ +	-	+ +
SECr - 3	+ +	+ +	+ +	-	+ +	+ +	+ +	-	+ +
SECr - 4	+	+	+	-	+ +	-	+ +	-	+ +
SECr - 5	+ +	+ +	+ +	-	+ +	+ +	+ +	-	+ +
SECr - 6	+ +	+ +	+ +	-	+ +	+ +	+ +	-	+ +
SECr - 7	+ +	+ +	+ +	-	+ +	+ +	+ +	-	+ +
SECr - 8	+ +	+ +	+ +	-	-	+ +	+ +	-	+ +
SKr - 9	-	+ +	+ +	W^{+}	-	+ +	+ +	-	+ +
SECr - 10	-	+ +	+ +	-	+	+ +	+ +	-	+ +

+ +, strongly positive; +, positive; W + weak positive; -, negative.

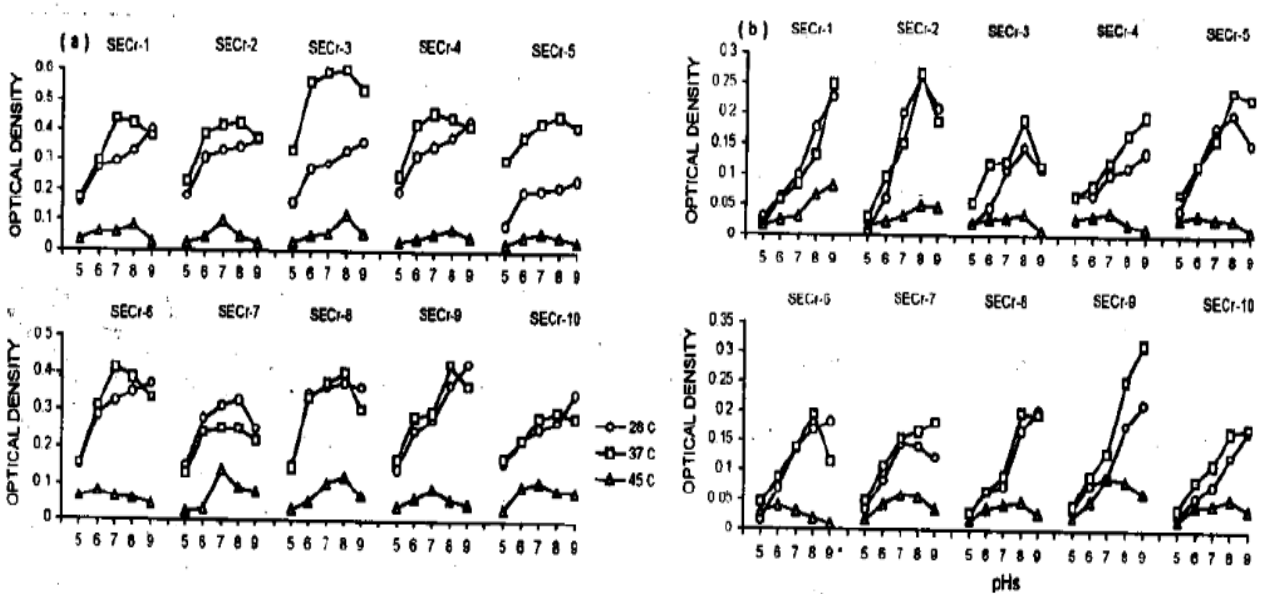

Fig. 2: Influence of varying pHs in combination with temperatures on growth of Cr (VI) resistant bacterial strains both in the absence (a) and presence (b) of 10 mg ml^{-1} of chromate salt. The cells were grown in nutrient broth at desirable pH and temperature for 24 hours. The bacterial growth was monitored at 600 nm

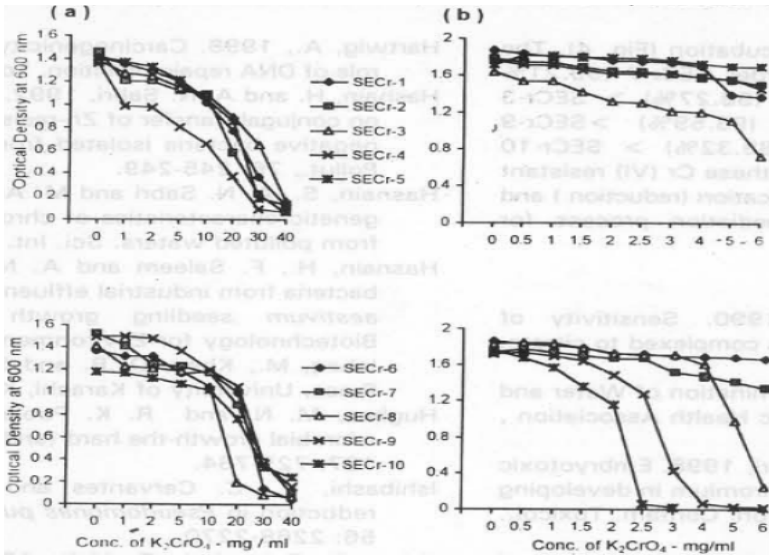

Fig. 3: Effect of varying concentrations of K_2CrO_4 on the growth of Cr (VI) resistant bacterial strains in nutrient broth (a) and M9 minimal medium (b). Bacterial cells were grown in nutrient broth or M9 medium supplemented with desirable canc. of K_2CrO_4 at 37°C for 24 hours. Growth was mortared at 600 nm

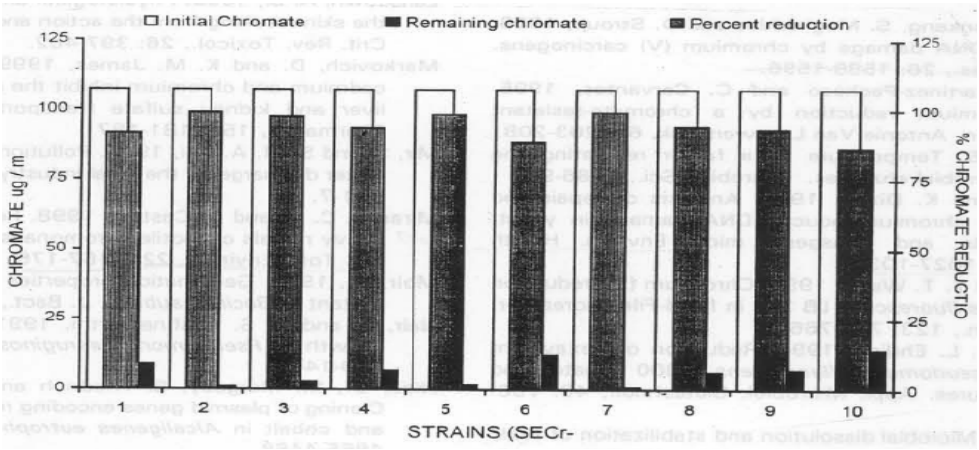

Fig. 4: Hexavalent chromium (chromate) reduction by Cr (VI) resistant bacterial strains isolated from effluents of electroplating industries after 24 hours of incubation

$FeCl_3$, $MnSO_4$, (except SECr-9), $NiCl_2$, $Pb(NO_3)_2$ and $ZnSO_4$ (Table 2). All the strains, however, were sensitive to $HgCl_2$. Pleiotropic metal resistances may be due to common mechanisms required for tolerance and resistance to these metals, a regulatory, factor, or common operon (Nies *et al.*, 1987). Schneider and Schweisfurth (1991) also reported the occurrence of pleiotropic metal resistances in bacteria isolated from polluted water. The Cr (VI) resistant strains were checked for resistances against various antibiotics and their resistance profile is shown in Table 2. These strains conferred resistant to ampicillin (except SECr-9, SECr-10), cefradine, cefadroxil, chloramphenicol (except SECr-8, SECr-9), kanamycin (except SECr-4) and tetracycline while they were sensitive to cyprofloxacin, doxycilline and streptomycin. Pleiotropic antibiotic resistances have been reported by many workers (Hasnain and Sabri, 1992; Yasmin *et al.*, 1997), *Aeromonas* isolates recovered by Miranda and Castillo (1998) from different polluted water sources were also resistant to many antibiotics. They concluded that highly polluted waters showed higher antibiotic multiresistances than moderately polluted water. Since trivalent Cr is much less toxic than hexavalent Cr, therefore, reduction of Cr (VII to Cr (III) represents an important means by which Cr toxicity is reduced and removal of Cr is facilitated (Deleo and Ehrlich, 1994).

Reduction of Cr(VI) has been reported by a variety of bacterial strains under a number of conditions (Deleo and Ehrlich, 1994; Fude *et al.*, 1994; Campos *et al.*, 1995; Turick *et al.*, 1996; Garbisu *et al.*, 1998; Tucker *et al.*, 1998). There is evidence for both aerobic and anaerobic Cr (VI) reduction systems with different microorganisms. Hexavalent Cr resistant strains were evaluated for their Cr (VI) reduction potential in aerobic mode. All these strains at initial chromate concentration of 107 μg ml^{-1}, reduced chromate

from 86.45% to 99.21% after 24 hours incubation (Fig. 4). The strains reduced chromate in descending order SECr-7 (99.21% reduction)> SECr-2 (98.97%) > SECr-5 (98.27%) > SECr-3 (97.33%) >SECr-8 494.04%) >SECr-4 (93.59%) >SECr-9 (93.22%) >SECr-1 (91.59%) >SECr-6 188.32%) > SECr-10 (86.45%). These results clearly shows that these Cr (VI) resistant strains have great potential for Cr (VI) detoxification (reduction) and can be utilized for developing a bioremediation process for contaminated Cr.

References

APHA., 1989. Standard Methods for the Examination of Water and Waste Water. 17th Edn., American Public Health Association, Washington, DC.

Al-Aoukaty, A. and V.D. Appanna, 1990. Sensitivity of *Pseudomonas syringae* to various metals complexed to citrate. Microb. Lett., 45: 105-111.

Asmatullah, S.N. and A.R. Shakoori, 1998. Embryotoxic and teratogenic effects of hexavalent chromium in developing chicks of Gallus domesticus. Bull. Environ. Contam. Toxicol., 61: 281-288.

Benschoter, A.S. and L.O. Ingram, 1986. Thermal tolerance of *Zymomonas mobilis*: Temperature-induced changes in membrane composition. Applied Environ. Microbiol., 51: 1278-1284.

Bose, R.N., B.S. Fonkeng, S. Moghaddas and D. Stroup, 1998. Mechanisms of DNA damage by chromium(V) carcinogens. Nucl. Acids Res., 26: 1588-1596.

Campos, J., M. Martinez-Pacheco and C. Cervantes, 1995. Hexavalent-chromium reduction by a chromate-resistant *Bacillus* sp. strain. Antonie Van Leeuwenhoek, 68: 203-208.

Chaloupka, J., 1985. Temperature as a factor regulating the synthesis of microbial enzymes. Microbial. Sci., 2: 86-90.

Cheng, L., S. Liu and K. Dixon, 1998. Analysis of repair and mutagenesis of chromium-induced DNA damage in yeast, mammalian cells and transgenic mice. Environ. Health Perspect., 106: 1027-1032.

Chirwa, E.M.N. and Y.T. Wang, 1997. Chromium(VI) reduction by *Pseudomonas fluorescens* LB300 in fixed-film bioreactor. J. Environ. Eng., 123: 760-766.

Deleo, P.C. and H.L. Ehrlich, 1994. Reduction of hexavalent chromium by *Pseudomonas fluorescens* LB300 in batch and continuous cultures. Applied Microbiol. Biotechnol., 40: 756-759.

Francis, A.J., 1990. Microbial dissolution and stabilization of toxic metals and radionuclides in mixed wastes. Experientia, 46: 840-851.

Fude, L., B. Harris, M.M. Urrutia and T.J. Beveridge, 1994. Reduction of Cr(VI) by a consortium of sulfate-reducing bacteria (SRB III). Applied Environ. Microbiol., 60: 1525-1531.

Fujie, K., H.Y. Hu, X. Huang, Y. Tanaka, K. Urano and H. Ohtake, 1996. Optimal operation of bioreactor system developed for the treatment of chromate wastewater using *Enterobacter cloacae* HO-1. Water Sci. Technol., 34: 173-182.

Garbisu, C., I. Alkorta, M.J. Llama and J.L. Serra, 1998. Aerobic chromate reduction by *Bacillus subtilis*. Biodegradation, 9: 133-141.

Gerhardt, P., R.G.E. Murray, W.A. Wood and N.R. Krieg, 1994. Methods for General and Molecular Bacteriology. 2nd Edn., American Society for Microbiology, Washington, DC., ISBN-10: 1555810489.

Hartwig, A., 1998. Carcinogenicity of metal compounds: Possible role of DNA repair inhibition. Toxicol. Lett., 102-103: 235-239.

Hasnain, H. and A.N. Sabri, 1992. Effects of temperature and pH on conjugal transfer of zinc-resistant plasmids residing in Gram-negative bacteria isolated from industrial effluents. Environ. Pollut., 76: 245-249.

Hasnain, S. and M. Abbas, 1997. Some growth and genetic characteristics of chromium resistant *Bacillus* strains from polluted waters. Sci. Int., 9: 87-94.

Hasnain, S., F. Saleem and A.N. Sabri, 1993. Lead Resistant Bacteria from Industrial Effluents and their Effects on the *Triticum aestivum* Seedling Growth under Lead Stresses. In: Biotechnology for Environment and Agriculture, Ahmad, N., M. Ishaq, Y. Khan and F. Sammar (Eds.). CMG Publications, Pakistan, pp: 193-205.

Hughes, M.N. and R.K. Poole, 1991. Metal speciation and microbial growth-the hard (and soft) facts. Microbiology, 137: 725-734.

Ishibashi, Y., C. Cervantes and S. Silver, 1990. Chromium reduction in *Pseudomonas putida*. Applied Environ. Microbiol., 56: 2268-2270.

Krieg, N.R. and J.G. Holt, 1984. Bergey's Manual of Systematic Bacteriology. 1st Edn., Vol. 1, Williams and Wilkins Co., Baltimore, MD., USA., ISBN: 0-683-04108-8, Pages: 964.

Lansdown, A.B., 1995. Physiological and toxicological changes in the skin resulting from the action and interaction of metal ions. Crit. Rev. Toxicol., 25: 397-462.

Markovich, D. and K.M. James, 1999. Heavy metals mercury, cadmium and chromium inhibit the activity of the mammalian liver and kidney sulfate transporter sat-1. Toxicol. Applied Pharmacol., 154: 181-187.

Mir, S. and S.M.A. Hai, 1999. Pollution due to hazardous waste-water discharge by the local industry and its control. Sci. Vision, 4: 1-7.

Miranda, C.D. and G. Castillo, 1998. Resistance to antibiotic and heavy metals of motile aeromonads from Chilean freshwater. Sci. Total Environ., 224: 167-176.

Moir, A., 1981. Germination properties of a spore coat-defective mutant of *Bacillus subtilis*. J. Bacteriol., 146: 1106-1116.

Nair, S. and V.S. Krishnamurthi, 1991. Effect of chromium on growth of *Pseudomonas aeruginosa*. Indian J. Exp. Biol., 29: 140-144.

Nies, D., M. Mergeay, B. Friedrich and H.G. Schlegel, 1987. Cloning of plasmid genes encoding resistance to cadmium, zinc and cobalt in *Alcaligenes eutrophus* CH34. J. Bacteriol., 169: 4865-4868.

Office of Technology Assessment, 1984. Protecting the nation's ground water from contamination. OTA-0-233, Office of Technology Assessment, Washington, DC.

Ohtake, H. and S. Silver, 1994. Bacterial Detoxification of Toxic Chromate. In: Biological Degradation and Bioremediation of Toxic Chemicals, Chaudhry, G.R. (Ed.). Dioscorides Press, Portland, pp: 403-415.

Patterson, D. and D. Gillespie, 1972. Effect of elevated temperatures on protein synthesis in *Escherichia coli*. J. Bacteriol., 112: 1177-1183.

Proctor, D.M., M.M. Fredrick, P.K. Scott, D.J. Paustenbach and B.L. Finley, 1998. The prevalence of chromium allergy in the United States and its implications for setting soil cleanup: A cost-effectiveness case study. Regul. Toxicol. Pharmacol., 28: 27-37.

Rahman, A. and U. Stall, 1996. Hazardous waste management for electroplating industries in Pakistan. Sci. Technol. Dev., 15: 34-40.

Sabri, A.N., M. Abbas and S. Hasnain, 1993. Growth characteristics of mercury resistant pseudomonads from industrial effluents and some factors influencing conjugal transfer of a plasmid. Sci. Int., 5: 51-58.

Schneider, S. and R. Schweisfurth, 1991. Species and genera of enterobacteriaceae in river neckar and after river bank filtration and their resistance patterns to antibiotics and heavy metal salts. Water Sci. Technol., 24: 315-320.

Singh, J., D.L. Carlisle, D.E. Pritchard and S.R. Patierno, 1998. Chromium-induced genotoxicity and apoptosis: Relationship to chromium carcinogenesis (review). Oncol. Rep., 5: 1307-1318.

Strnadova, M., M. Hecker, L. Wolfel, H. Mach and J. Chaloupka, 1991. Temperature shifts and sporulation of *Bacillus megaterium*. Microbiology, 137: 787-795.

Sugiyama, M., 1994. Role of paramagnetic chromium in chromium(VI)-induced damage in cultured mammalian cells. Environ. Health Perspect., 102: 31-33.

Towil, L.E., C.R. Shriner, J.S. Drury, A.S. Hammons and J.W. Holleman, 1978. Reviews of the environmental effects of pollutants: III Chromium, US. Environmental Protection Agency Report. 600/1-78-023, 287, Washington DC.

Tucker, M.D., L.L. Barton and B.M. Thomson, 1998. Reduction of Cr, Mo, Se and U by *Desulfovibrio desulfuricans* immobilized in polyacrylamide gels. J. Ind. Microbiol. Biotechnol., 20: 13-19.

Turick, C.E., W.A. Apel and N.S. Carmiol, 1996. Isolation of hexavalent chromium-reducing anaerobes from hexavalent-chromium-contaminated and noncontaminated environments. Applied Microbiol. Biotechnol., 44: 683-688.

Turick, C.E., C.E. Camp and W. A. Apel, 1997. Reduction of $Cr(6^{+})$ to $Cr(3^{+})$ in a packed-bed bioreactor. Applied Biochem. Biotechnol., 63: 871-877.

Wang, Y.T. and C. Xiao, 1995. Factors affecting hexavalent chromium reduction in pure cultures of bacteria. Water Res., 29: 2467-2474.

Yasmin, A., S. Afrasayab and S. Hasnain, 1997. Mercury resistant bacteria from effluents of paint factory: Characterization and mercury uptake ability. Sci. Int., 9: 315-320.

Zhitkovich, A., V. Voitkun, T. Kluz and M. Costa, 1998. Utilization of DNA-protein cross-links as a biomarker of chromium exposure. Environ. Health Perspect., 106: 969-974.