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BIOLOGICAL CHROMIUM REDUCTION AT LOW pH: A PRELIMINARY STUDY

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Abstract: Chromium (Cr^{+6}) tolerant acidophilic bacteria were isolated from tanneries' effluents, optimized for growth conditions and biochemically characterized. Two of the isolates were assessed for their Cr^{+6} reduction potential. It was found that the isolates No. 22 and 25 efficiently reduced 750 µg Cr^{+6}/ml of the medium within 4 days of incubation. These bacteria can be considered important for bioremediation of metal rich acidic environments or industrial effluents. These microbes may play role for developing new strategies for remediation of heavy metals' polluted wastewaters characterized by low pH.

Keywords: Acidophilic bacteria, bioremediation, metal detoxification, tannery effluents.

INTRODUCTION

Anthropogenic activities especially of industrial level have contaminated aquatic and terrestrial environments with omnifarious heavy metals. Several industries are considered to be the major culprits of heavy metals' pollution (Idris *et al*., 2007; Malla *et al*., 2007). It is well known that metals including radioactive ones are transferred to animal and human beings through food chains and exert harmful effects (Ghafoor *et al*., 1995; Qazi and Jafri, 1996). The principal health hazards of various metals and their anthropogenic sources of generation have been reported by a number of researchers (Apostoli *et al*., 2000; Martinez and Motto, 2000; Goyer and Clarkson, 2001; Manahan, 2003; Landis and Yu, 2004; Campbell, 2006; Scrag, 2006). Hexavalent chromium (Cr^{+6}) is widely used in tanning process as a leather softening and strengthening agent (Cary, 1982). Despite of its crucial role as an

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essential trace mineral in carbohydrate and fat metabolism, exposure to chromium in higher concentrations is highly clastogenic, carcinogenic, mutagenic and tetratogenic (Anderson *et al.,* 1983; Chakrabortry *et al.,* 1992; Lansdown, 1995; Kaats *et al.,* 1996). It has been reported to cause cancer of intestines, lungs and tonsils in tannery workers (IARC, 1981; Sweeney *et al.,* 1985; Coggon *et al.,* 1986).

Removal of metals from industrial effluents is much necessary before discharging them to the environment. A number of physicochemical treatment methods are used for the removal of metals, majority of which involve energy expenditure and chemicals (Fatin-Rouge *et al*., 2006; El-Samrani, 2008; Aguado *et al*., 2009; Dizge, 2009). Such methods have thus been declared environmentally non-compatible by various researchers due to low treatment efficiency, complicated operation, high operational cost and generation of secondary pollutions (Saeed and Iqbal, 2003; Rocha *et al*., 2009). While, biological methods of metals' removal have gained importance for their better performance, low cost and environmentally compatible natures (Akhtar and Mohan, 1995; Puranik and Pakniker, 1999). Keeping in view the facts, biological detoxification / treatment of metal containing industrial effluents attracts special attention.

Biochemical studies have shown that a broad spectrum of microbes can reduce soluble, toxic Cr^{+6} to insoluble, less toxic Cr^{+3} (Wang and Shen, 1995). The present study was aimed at screening acidophilic Cr^{+6} reducing bacteria already isolated and preserved from the tanneries' effluents of the city Kasur, Pakistan. The wastewater of the study area has been found to contain 3 to 6 µg/ml of Cr^{+6} with pH values ranging from 5 to 9 (Qazi *et al.,* 1997). The acidophilic bacteria were employed for the treatment of Cr^{+6} rich waters. Such bacteria are expected to fill the gap between biological and chemical processes meant for detoxification of metal contaminated sites.

MATERIALS AND METHODS

Six acidophilic bacterial strains, preserved in Microbial Biotechnology Laboratory, Department of Zoology, University of the Punjab (Quaid-e-Azam campus), Lahore were revived for this study.

Optimization of growth conditions

Growth was revived in LB broth and the bacteria were optimized for oxygen demand (aerobic, anaerobic natures), effect of pH (5.0, 5.5 and 6.0), inoculum size (1, 5 and 10%) and temperature (25, 37 and 50^{o}C).

Biochemical and cellular characterization

Overnight grown bacterial cultures were processed for catalase, Koser's citrate, MacConkey agar, oxidase, starch utilization and Voges–Proskauer tests. The bacterial isolates were also stained for capsular, endospore and Gram's reaction (Benson, 1994).

Bacterial growth in the presence of Cr^{+6}

Three concentrations of Cr^{+6} in L.B broth were prepared using $K_2Cr_2O_7$ in such a way that the quantity if Cr^{+6} was 0.25, 0.5 and 0.75 mg/ml in first, second and third concentration, respectively. The bacterial strains were cultivated in control (LB broth without addition of Cr^{+6}) as well as the experimental media under respective growth optima. Then at different prescribed intervals, 5 ml of bacterial culture was taken from each flask and processed for photometric growth assessment at 600 nm. Thereafter, the sampled bacterial cultures were centrifuged at 4000 rpm for 10 min. Supernatant was separated and pH measured. Following the pH measurement it was stored in a freezer for subsequent Cr^{+6} estimation.

Estimation of Cr^{+6}

Chromium (VI) was estimated after Petrilli and Deflora (1977). To prepare a standard curve, different concentrations of $K_2Cr_2O_7$ were prepared in the LB broth. First of all 0.5 ml of each solution was taken in 250 ml conical flask and 24.5 ml distilled water was added to each flask. Then 1.5 ml of concentrated H_2SO_4 was added to attain a pH range of 0.45-0.5. Volume in each flask was then made 50 ml by adding distilled water. To the mixture, 10 ml of freshly prepared diphenyl carbazide reagent (0.5% w/v in acetone) was added. Contents of the flask were mixed well and kept at room temperature for 10 min. Then O.D. of the sample was measured at 540 nm taking glass distilled water as reference. A standard curve prepared from the experiment was used to estimate differences between the levels of Cr^{+6} prior and after the bacterial.

RESULTS AND DISCUSSION

All the six bacterial strains showed optimum growth at 30°C aerated in incubations. The strains No. 22, 25, 26 and 27 had optimum pH 6.0, while, strains No. 23 and 24 showed best growth at pH 5.5 (Fig 1, Table I). Higher concentrations of Cr^{+6} *i.e.,* 500 and 750 µg/ml suppressed growth of the bacteria strain No. 22 up to 64 hours of incubation. However, the growth showed a shooting increase from 64 hours onwards to the end of experiment. At higher concentrations of Cr^{+6}, pH of the medium decreased gradually around 64 hours of incubation with accompanying significant increase in the growth. Regarding the metal detoxification potential up to 100, 96 and 97% reductions of Cr^{+6} were recorded for the media amended with 250, 500 and 750 µg of Cr^{+6}/ml, respectively.

Growth of the bacterial strain No. 25 was slightly suppressed by higher concentrations of the metal and it was interesting to note that pH of the culture fluids didn't change much right from beginning to the end of the experiment. This bacterial strain appeared more efficient in reducing 250 and 500 µg/ml of Cr^{+6} within 16 hours, while, the complete reduction of Cr^{+6} was recorded at the end of sampling period. Around 100% reductions of Cr^{+6} concentrations tested at 88 hours are meaningful for bioremediation of heavy metal contaminated sites (Table II). However, failure of 100% reduction of 750 µg/ml of the metal by strain No. 22 indicates the necessity for regulating the effluents dilutions that can be bioprocessed by the indigenous microbiota to detoxify the pollutants or in a designed bioreactor. The present study was taken in search of microbes of extreme environment for biotechnological processing (Schmieman *et al.,* 1997).

The bacteria reported to be resistant to chromate reduce chromate (Cervantes and Silver, 1992). Chromate resistance was once mainly thought to be based on chromate efflux. However, recent data suggested that both efflux and reduction processes are involved (Peitszch *et al.,* 1998). Amongst the benefit using the extremophilic bacteria for biotechnological processes such as bioremediation, the mentionable fact is, many effluents are characterized by high or low pH, thermal pollution and high contents of other substances. To detoxify one or more notorious pollutants in such an environment through eubacteria first requires rendering such conditions near to the optima levels for the microbes for the remediation processes.

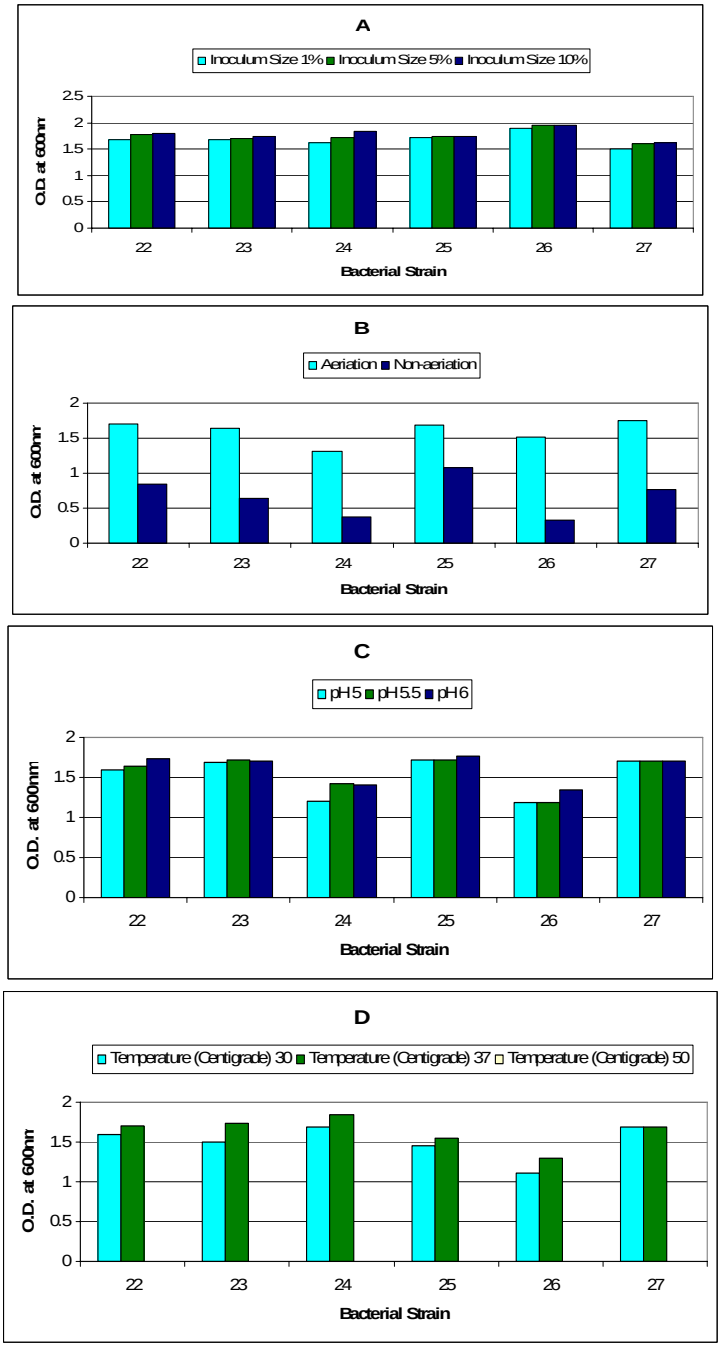

Figure 1 (A-D): Growth conditions optimization of the bacterial isolates.

This involves further expenditure and may make in various conditions the processes economically not feasible. The bacterial isolates characterized in the present study have potential to reduce Cr^{+6} in effluents characterized with low pH. However, cheaper growth nutrients instead of bacteriological media used in this investigation are to be tested to reduce the cost of the process.

Table I: Biochemical and cellular characterization of the bacterial strains

Characteristics	Strain code					
	22	23	24	25	26	27
Catalase	+ve	+ve	+ve	+ve	+ve	+ve
Koser's Citrate	–ve	+ve	–ve	–ve	+ve	+ve
MacConkey Agar	+ve	+ve	–ve	+ve	–ve	+ve
Oxidase	+ve	+ve	+ve	+ve	–ve	+ve
Starch	+ve	+ve	+ve	+ve	+ve	+ve
Voges–Proskauer	+ve	+ve	+ve	+ve	+ve	+ve
Capsule	–ve	–ve	–ve	–ve	–ve	–ve
Endospore	–ve	–ve	–ve	+ve	+ve	+ve
Gram's Staining	–ve	–ve	–ve	+ve	–ve	+ve

Table II: Bacterially mediated chromium (Cr^{+6}) reduction at different incubation hours

Strain code	Conc. of Cr^{+6}	Incubation Period (hours)											
		16			40			64			88		
		A	B	C	A	B	C	A	B	C	A	B	C
22	250	250 ±0.02	0.72 ±0.00	7.19 ±0.01	250 ±0.02	1.43 ±0.01	7.90 ±0.01	250 ±0.01	1.66 ±0.01	8.26 ±0.01	250 ±0.02	1.78 ±0.01	7.89 ±0.02
	500	478 ±0.01	0.19 ±0.00	5.92 ±0.02	489 ±0.01	0.20 ±0.01	5.96 ±0.02	492 ±0.01	0.33 ±0.01	6.04 ±0.03	500 ±0.02	1.42 ±0.07	5.56 ±0.01
	750	714 ±0.01	0.09 ±0.01	6.20 ±0.01	722 ±0.02	0.09 ±0.01	6.20 ±0.01	734 ±0.01	0.18 ±0.00	5.85 ±0.02	747 ±0.01	0.56 ±0.02	5.60 ±0.05
25	250	250 ±0.00	1.17 ±0.02	7.65 ±0.03	250 ±0.02	1.54 ±0.01	8.39 ±0.01	250 ±0.01	1.65 ±0.01	8.33 ±0.03	250 ±0.00	1.86 ±0.01	8.51 ±0.01
	500	495 ±0.02	1.02 ±0.01	7.58 ±0.01	499 ±0.01	1.39 ±0.02	8.20 ±0.03	500 ±0.01	1.59 ±0.00	8.41 ±0.02	500 ±0.00	1.81 ±0.01	8.32 ±0.01
	750	721 ±0.06	0.74 ±0.01	7.56 ±0.02	738 ±0.01	1.30 ±0.03	8.17 ±0.06	750 ±0.01	1.54 ±0.01	8.46 ±0.01	750 ±0.00	1.75 ±0,01	8.54 ±0.01

Values are means of three replicates ±SEM

Abbreviations used: A, Cr^{+6} reduction (mg); B, Growth (O.D) at 540 nm; C, pH

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