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Reduction of Toxic Hexavalent Chromium by *E. coli*

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Abstract: Reduction of toxic hexavalent was studied by using four resistant strains of *E. coli* ASU 3, 7, 8 and 18 isolated from wastewater of EL-Malah canal located in Assuit city, Egypt. They showed relatively high minimal inhibitory concentrations (MIC) and found to be plasmid mediated with 65 and 27 Kb. *E. coli* ASU 7 represents the best with high resistance and reducing power of Cr (VI), so it may be a suitable candidate for bioremediation. Alternation of protein profile in SDS-PAGE of the above strain was carried out under different concentrations of chromium stress.

Kew words: Chromate reduction · *E. coli* · Plasmid isolation and SDS-PAGE

INTRODUCTION

Heavy metals found in wastewaters are harmful to the environment and their effects on biological systems are very severe. Chromium is one of the most widely used metals in industry, such as steel production, alloy preparation, wood preservation, leather tanning, metal corrosion inhibition, paints pigments, metal plating, tanning, electroplating, steel manufacture and other industrial applications [1-3]. Chromium (Cr) is an essential trace element for all living organisms. Its valence state ranged from -2 to +6. Trivalent chromium is necessary for fat and glucose metabolism and proper functioning of insulin [4]. Hexavalent chromium is easily soluble and 100-fold more toxic than trivalent one.

Hexavalent chromium it has been recognized as one of the most dangerous environmental pollutants due to its ability to cause mutations, irritation, corrosion of the skin and respiratory tract to most microorganisms; it also causes lung carcinoma in humans [5-7]. Efficient and cheap treatments for heavy metal removal and reusing of spent metals from wastewater need to be developed, microbe based technologies can provide an alternative to conventional methods for metal removal [8]. The reduction of Cr (VI) to Cr (III) is therefore an attractive and useful process for remediation of Cr (VI) pollution; so many technologies have accordingly received much more concerns [5].

The presence of Cr (VI) in the environment plays a selective pressure on microflora and possess resistance to high levels while others are sensitive [9]. The bacterial chromate resistance is generally combined to plasmids but it can also be coupled to chromosomal DNA [10-11].

Microorganisms can play an important role in the removal of hexavalent chromium from the polluted sites [12]. A wide variety of bacteria have been reported to reduce hexavalent to trivalent chromium under aerobic and anaerobic conditions [13-17]. Biotransformation of chromate by chromium resistant bacteria (CRB) offers an economical as well as eco-friendly option for chromate detoxification and bioremediation [18]. The main advantages of using bacterial Cr(VI) reduction are that it does not require high energy input nor toxic chemical reagents and the possibility of using native and non-hazardous strains [19].

The objective of the present work is to characterize and evaluate plasmid mediated chromate resistant *E. coli* with high potentialities to reduce hexvalent chromium to trivalent one and analysis of their alternation of protein profile by SDS-PAGE under chromium stress.

MATERIALS AND METHODS

Determination Physicochemical Properties of Water Samples: Different polluted water samples were collected in sterile glass bottles from different sites in El-Mlalah Canal, Assiut City (Egypt), it is far about 3 km from the main River Nile in the west site of Assiut City and exposed to domestic sewage disposal &

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industrial effluent. Temp, pH and Electrical conductivity were determined by water checker model Horiba U-10. Heavy metals were determined according to Elith and Garwood [20].

Isolation of *E. coli:* *E. coli* was enumerated from wastewater samples by Most Probable Number (MPN) techniques using lactose broth medium [21]. Positive presumptive tubes show acid and gas when incubated at 37 °C for 48 hours.

Biochemical Identification: Various biochemical tests (Indole, Methyl test, Voges-Proskauer, Citrate, Hydrogen sulfide on TSI, Urease, Motility, Gelatin liquefaction at 22°C, KCN, Lactose, nitrate reduction and Oxidase test) were done according to Bergey's Manual of Systematic Bacteriology [22].

Evaluation of Chromium Resistance for *E. coli* Strains: Minimal Inhibitory Concentration (MIC) for hexa- and trivalent chromium were registered on agar plates of tris minimal agar medium using agar dilution methods and were confirmed in broth medium, for broth medium a 5 ml of tris minimal medium containing different concentrations of $CrCl_3.6H_2O$ (0-300 ppm) and K_2CrO_4 (0-25 ppm) inoculated with 200 µl of an 18 h old culture of the studied bacterial *E. coli* strain at 37°C for 2 days. The lowest concentration of heavy metals that completely preventing growth known as MIC [23].

Chromate Reduction in Liquid Medium: The chromate reduction capability of isolates was investigated under aerobic conditions in tris minimal broth medium amended with various concentrations of chromium ranged from 1 to 10 ppm at pH 7.0 [24]. Chromate reduction was suggested by Bopp and Ehrlich [25]. Broth medium inoculated with 200 µL of preculture with shaking at 150 rpm and 37°C for 48 h. Cr^{6+} added after 3hr of incubation and measured at different times intervals (3-48 h). Cells were centrifuged at 10.000 xg for 10 min then the supernatant was filtrated through filter papers (Whatman no 1) then chromate reduction was quantified by measuring the decrease in absorbance at 382 nm using a Cecil spectrophotometer.

Plasmid Isolation: Plasmid isolation of bacterial cells was carried out according to Birnboim and Doly [26] using alkaline lysis procedure, plasmid DNA size was determined by the LabImage 1D (2006) software program.

Plasmid Transformation: To confirm the plasmid-encoded resistance to chromium studied, competent cells of *E. coli* DH5α, sensitive to hexavalent chromium were transformed with respective plasmids using the standard chemical method [27]. The suspensions (100 µl) of transformed *E. coli* DH5α were plated on tris agar minimal media supplemented with 20 ppm of hexavalent chromium.

SDS-PAGE of Proteins: The protein patterns were analyzed using SDS-PAGE according to Laemmli [28] in the first dimension. Gels were stained overnight in 200 ml of Commassie brilliant blue R-250 solution. Destaining of protein was performed in 200 ml of destaining solution which composed of 250 ml methanol, 50 ml glacial acetic acid and 200 ml distilled water with gentle shaking.

Protein Analysis: The LabImage 1D (2006) program was used in molecular weight determination of proteins.

RESULTS AND DISCUSSION

Physicochemical Properties of Samples: The temperature, pH and Electrical conductivity corresponded to 23.7-27 °C; 7.9-8.12 and 0.34-0.35 ms/Cm, respectively. The concentration of chromium was expressed in mg/l and ranged between 0.38-0.88 mg/l. The results obtained exceeded the safe limit of WHO which is 0.05 mg/l of chromium [29].

Isolation of *E. coli* Isolates*:* *E. coli* was enumerated from wastewater samples by MPN techniques using lactose broth medium. Positive presumptive tubes were confirmed on Eosin Methlyene Blue (E.M.B) agar medium. Metallic green isolates were selected randomly, purified and preserved on nutrient agar for further studies.

Biochemical Identification: Bacteria are gram negative rods. Various biochemical characteristics of the strains were shown in Table 1. Based on the biochemical analysis, bacteria have been identified as *E. coli* according to Bergey's Manual of Systematic Bacteriology [22].

Evaluation of Chromium Resistance for *E. coli* Strains: The MIC of selected strains of *E. coli* ASU 3, 7, 8 and 18 were registered on tris minimal medium and confirmed

Table 1: Biochemical characterization of the isolates

Test	Isolate	Reaction ASU 3	ASU 7	ASU 8	ASU 18
Indole production		+	+	+	+
Methyl red		+	+	+	+
Voges-Proskauer		-	-	-	-
Citrate		-	-	-	-
Hydrogen sulfide on TSI		-	-	-	-
Urease		-	-	-	-
Motility		+	+	+	+
Gelatin liquefaction at 22°C		-	-	-	-
KCN, growth in		-	-	-	-
Lactose		+	+	+	+
$NO_3^- \rightarrow NO_2^-$		+	+	+	+
Oxidase		-	-	-	-

Table 2: Minimum inhibitory concentrations (MIC) for both Cr^{6+} and Cr^{3+} representing in ppm or mM of *E. coli* ASU 3, 7, 8 and 18 grown in tris broth minimal medium at 37 °C for 48 h

	MIC			
E. coli ASU	Cr^{6+} ppm	mM	Cr^{3+} ppm	mM
3	20	0.38	400	7.69
7	25	0.48	400	7.69
8	20	0.38	200	3.85
18	25	0.48	200	3.85

MIC: Defined as the lowest concentration of metal that completely preventing growth

in the above broth medium. The (MIC) measurements on solid medium were higher than those in liquid medium. This may be due to the condition of diffusion; complexation and availability of metals in liquid medium were different from those observed in solid medium.

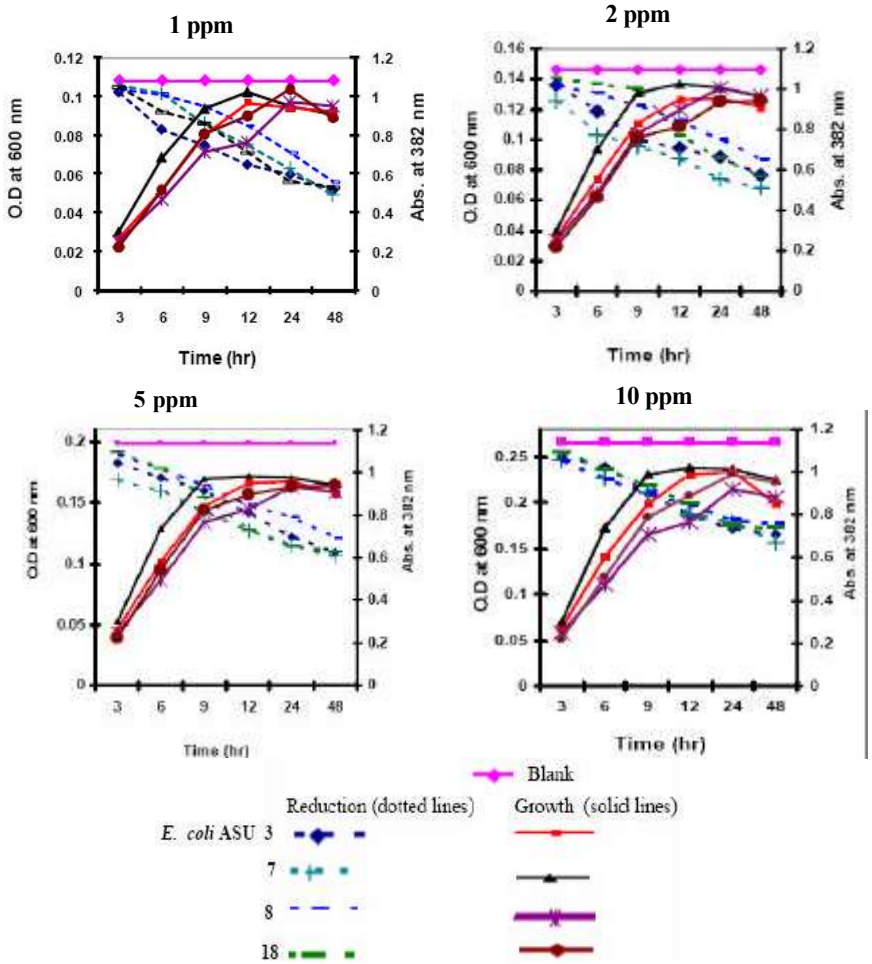

Fig. 1: Cr(VI) reduction (dotted Lines) and bacterial growth (solid lines) of four *E. coli* grown in tris minimal broth medium supplemented with different concentrations (1, 2, 5 and 10 ppm) of Cr^{6+}. Chromate reduction was followed by measuring decrease in absorbance at 382 nm; bacterial growth was monitored at O.D 600 nm

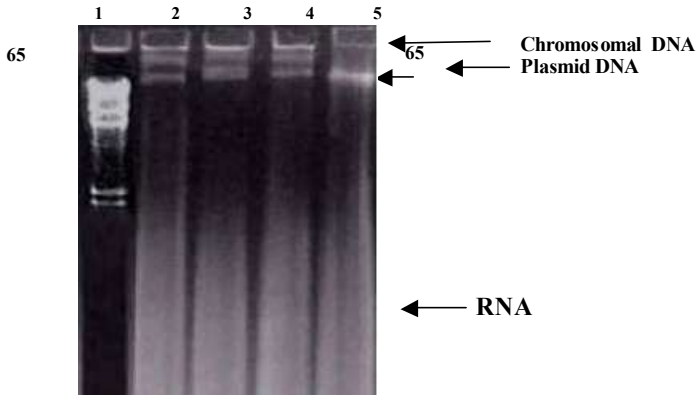

Fig. 2: Agarose gel (1%) Electrophoresis of four resistant strains of *E. coli* ASU 7, 3, 8 and 18 corresponding to lanes 2, 3, 4 and 5, respectively describing plasmid and chromosomal DNA, lane 1 λ (DNA marker) cleaved with Hind III.

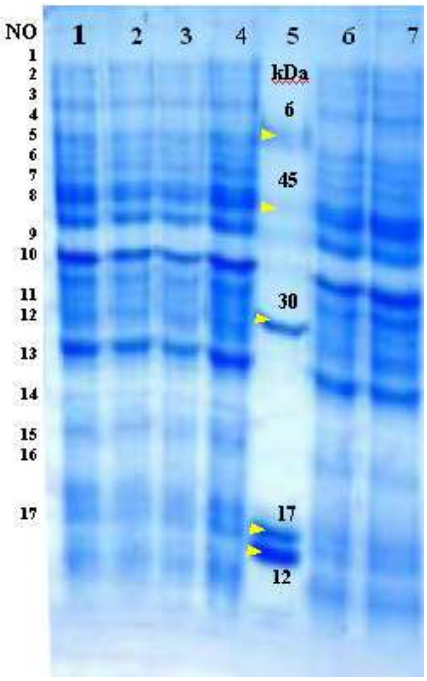

Fig. 3: Show the soluble protein patterns in (12 % SDS-PAGE gel stained in Coomassie blue) of whole cell lyste of *E. coli* ASU 7 grown in tris minimal broth medium and incubated at 37 °C for 24 hours. Lane 1-3 Cr^{6+} at concentrations 1, 5, 10 ppm; lane 4 control; lane 5 marker (in KDa); lane 6-7 Cr^{3+} at concentrations 20, 30 ppm; respectively

This suggests that organic matter component in the agar forming complexes with heavy metals e.g (Pb, Cd and Zn); thereby reducing the concentration of free metal ion. These observations are in agreement with Mergeay [24], Babich and Stotzky [30] and Hassen *et al*. [31].

The MIC comparison of chromium resistance strains of *E. coli* to both hexa and trivalent cations was given in Table 2. It explained that *E. coli* ASU 7 exhibited the highest level of chromium tolerance than other strains. Its values ranged from 0.48 to 7.69 mM for Cr^{6+} and Cr^{3+}, respectively. These results were higher than that obtained by Viti *et al* [10] reported that chromate resistant bacteria of *Pseudomonas sp* have MIC 0.2 mM of Cr^{6+}.

Chromate Reduction in Liquid Medium: Growth as well as chromate reduction was monitored with different concentrations ranged from 1 to 10 ppm of Cr(VI) at 37°C in aerobic conditions under shaking with (150 rpm) by four strains of *E. coli* as shown in Fig. 1. The rate of reduction increased with decreasing chromium concentration used. The strain *E. coli* ASU 7 showed the best chromate reduction strain than others. Its reduction values were 54.62, 53.42, 46.73 and 41.35% at concentrations 1, 2, 5 and 10 ppm of Cr(VI) after 48 h incubation respectively. The reduction in our results are more than that obtained by Pattanapipitpaisal *et al*. [32] they stated that *Microbacterium* MP30 has the ability to reduce Cr(VI) under anaerobic conditions at concentration corresponding to 0.1 ppm metal ion by 99.7 %.

Plasmid Content of Isolates: All chromate-resistant isolates were examined for the presence of plasmid DNA. Most strains have two plasmids with molecular weights 27 and 65 Kb except *E. coli* ASU 18 contains one plasmid with 27 Kb as shown in Fig. 2. These results are in agreement with Viti and Giovannetti [9] showed that the strain ChrC20 exhibited a plasmid with molecular size >55 kb and the strain ChrB20 showed two plasmids with molecular sizes <55 kb and about 55 kb.

Plasmid-specified chromate resistance has been also reported for both *Streptococcus Lactis* [33] and *Pseudomonas aeruginosa* [34]. They have approximately 10-fold resistant to chromate than in the plasmidless strain. [35-36] suggested that all heavy metal-resistant strains isolated from environments polluted with metals exhibited metal resistance determinants on plasmids.

Plasmid Transformation: To determine whether the resistance markers of *E. coli* ASU 7 on plasmid DNA, the transformation was carried on a recipient cell of *E. coli* DH5α. By comparing the growth results, it was found that the transformant strain of *E. coli* DH5α could grow on tris minimal medium containing 20 ppm of hexavalent chromium while the non-transformant can not.

SDS-PAGE of Proteins: The inducible response of metal stress (Cr^{6+} and Cr^{3+}) of *E. coli* ASU 7 was studied. The electrophoresis analyses in 12 % SDS-PAGE of whole cells lysate protein are summarized in Fig. 3. It was obvious that some sets of proteins were induced with molecular weights (77, 48, 15 and 13) under 1 ppm of Cr^{6+} stress. This group of proteins characterized with high intensity and may be responsible for chromate reduction. Our results are similar to that obtained by Thacker and Madamwar [37] they reported that protein with molecular weight 30 KDa was induced under stress of chromium and this may be associated with reduction of chromium.

In the present study, we isolated four resistant strains of *E. coli* with different potentialities to remediate or to reduce toxic hexavalent to trivalent one. Most of them were found to be plasmid mediated. *E. coli* ASU 7 showed the best chromate resistant bacteria and its resistance to be carried on plasmid DNA. SDS-PAGE showed induction of some sets of proteins under chromium stress.

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