

Reduction of Chromium(VI) by Locally Isolated *Pseudomonas* sp. C-171

Mujeeb UR RAHMAN¹, Shereen GUL², Mohammad Zahoor UL HAQ¹

¹PCSIR Laboratories, P.O. Box 387, Mastung Road, Quetta, Balochistan, PAKISTAN

²Department of Botany, Government Girls College, Quetta, Balochistan, PAKISTAN

Received: 27.10.2006

Abstract: A strain of *Pseudomonas* sp. C-171 capable of tolerating hexavalent chromium (Cr⁺⁶) up to 2000 ppm as potassium dichromate was isolated from domestic sewage. The Cr⁺⁶ reduction was checked by growing the isolated strain in a medium containing potassium dichromate as Cr⁺⁶ source. The rate of growth of *Pseudomonas* sp. C-171 decreased with the increase in Cr⁺⁶ concentration of the medium. The maximum rate of chromium reduction was observed during the log phase of bacterium growth. The reduction of hexavalent chromium was inoculum concentration dependent. The accumulation of chromium hydroxide around the bacterial cells and slight elongation of cells were observed.

Key Words: Hexavalent chromium, reduction, *Pseudomonas* sp. C-171, accumulation, chromium hydroxide, cell elongation

Introduction

Hexavalent chromium (Cr⁺⁶) is a common water pollutant and is toxic to most organisms. Cr⁺⁶ is highly soluble in water and forms divalent species, chromate (CrO₄⁻²) and dichromate (Cr₂O₇⁻²). Cr⁺⁶ is a strong oxidizing agent. As a result of redox reactions, Cr⁺⁶ is reduced to trivalent chromium Cr⁺³, which forms insoluble chromium hydroxide at neutral pH. In biological systems, the cellular membranes are permeable to Cr⁺⁶. Cr⁺⁶ passes through the cellular membrane and is reduced to Cr⁺³ in mitochondria and nuclei, as well as in cytoplasm. Although Cr⁺³ is impermeable to the membrane, Cr⁺³ generated inside the cell binds stably to protein and interacts with nucleic acid (1).

Microbial transformations for different metallic minerals have been reported by Chiong et al. (2) and Lebedeva and Lyalikova (3). The transformations include redox conversions of inorganic forms, inorganic to organic form and vice versa (4). Some microbial transformations enable the bacteria to increase their tolerance towards heavy metals (2). Bacterial reductions have been found in metallic minerals such as manganese (5), mercury (6) and selenite (7). The reduction of Cr⁺⁶ by sulfate reducing bacteria has been reported by Smith

(8), Shakoori et al. (9), Schmieman et al. (10), and Rahman and Gul (11). Many other researchers (12-15) have reported that bacterial strains such as *Pseudomonas* sp., *Enterobacter* sp. and *Desulfovibrio* also have the ability to transform Cr⁺⁶ to Cr⁺³. In bacteria when the reduction of Cr⁺⁶ takes place at the cell surface or by bacterial metabolic products such as H₂S, Cr⁺³ is observed to form extracellular insoluble chromium hydroxides, which subsequently precipitate in the medium. Thus Cr⁺⁶ is detoxified and immobilized. This unique ability of bacteria to reduce Cr⁺⁶ may be of use as a promising means of bioremediation of Cr⁺⁶ (16).

In the present study, a Cr⁺⁶ tolerant bacterial strain was isolated from domestic sewage and the effects of Cr⁺⁶ on its growth and Cr⁺⁶ reducing ability were determined.

Materials and Methods

Chromium compound

Potassium dichromate (K₂Cr₂O₇) A.R. grade from Sigma was used as the source of hexavalent chromium (Cr⁺⁶).

Isolation of Cr⁺⁶ resistant bacterium

The Cr⁺⁶ resistant bacteria were isolated by agar plate technique. The basal medium (g/l) Polypeptone 5, meat extract 5, NaCl 5, glucose 5, and agar 20 was used for the isolation of the bacterium. The pH of the medium was adjusted to 7.0 with 0.1 N NaOH. The chromium compound was dissolved in distilled water, sterilized, and added to the medium in 250 ppm concentration prior to plating. These plates were then inoculated with a suitable dilution of sewage. The colonies that appeared were transferred to agar plates having basal medium containing 500, 1000, 1500, and 2000 ppm Cr⁺⁶ for screening of tolerant strains.

Identification of hexavalent chromium resistant bacterium

Taxonomic studies of the selected Cr⁺⁶ resistant bacterium were carried out according to Buchanan and Gibbons (17). The isolated bacterium was identified by the following taxonomic studies:

1. Morphological characteristics (shape and size, gram reaction, motility)
2. Cultural characteristics (nutrient agar colonies, slant culture, stab culture)
3. Physiological characteristics (gelatin liquification, milk clotting, indol, H₂S, NH₃ production, Voges-Proskauer reaction, Methyl red test, starch hydrolysis, catalase and urease).

Inoculum Preparation

The basal medium without agar was used for the preparation of inoculum. Sterilized 50 ml of medium in a 250 ml conical flask was inoculated with a loop of isolated bacteria and incubated at 30 °C on rotatory shaker rotating at 150 rpm for 18 h.

Bactericidal resistance to chromium compounds

The bactericidal action of the chromium compound was studied at a concentration of 3000 ppm for CrCl₃ (trivalent, pH 5.0) and K₂Cr₂O₇ (hexavalent, pH 7.0); 1 ml of bacterial inoculum having $\approx 1.5 \times 10^5$ cells was inoculated with 1 ml of 6000 ppm solution of the respective chromium compound and incubated at 30 °C. The samples were drawn after an interval of 0, 4, 8, 12,

16, and 20 min. A suitably diluted 0.5 ml sample was transferred to nutrient agar plates and incubated at 30 °C for 24 h. The number of colonies that appeared on nutrient agar was counted and the surviving cells were calculated as follows:

Number of surviving cells per ml = Number of colonies on plate $\times 2 \times$ Dilution factor

The total number of killed bacteria was calculated by the following formula:

Number of killed cells per ml = Initial number of cells – Number of surviving cells

Effect of hexavalent chromium on growth of the isolated bacterium

These studies were carried out by inoculating medium containing 250, 500, 1000, and 1500 ppm Cr⁺⁶ as K₂Cr₂O₇ with 30% inoculum V/V. The medium was incubated at 30 °C with shaking at 150 rpm. The samples were drawn at regular intervals of 6 h up to 60 h for growth measurement (optical density at 600 nm (OD₆₀₀)). The optical density of the medium was measured by spectrophotometer (U2000, Hitachi).

Reduction of hexavalent chromium

The studies on the reduction of hexavalent chromium were carried out for 1500 ppm Cr⁺⁶ in the basal medium without agar. The medium was inoculated by 20% V/V 18-h-old inoculum and incubated at 30 °C with shaking at 150 rpm. The samples were drawn at regular intervals of 12 h up to 288 h for the estimation of reduced hexavalent chromium.

Effects of inoculum concentration on reduction of hexavalent chromium

The effects of inoculum concentrations were studied by adding inoculum 10%, 20%, and 30% V/V to the medium containing 1500 ppm Cr⁺⁶ as K₂Cr₂O₇. The samples were drawn at regular interval of 12 h up to 96 h for estimation of reduced Cr⁺⁶.

Analytical methods

Hexavalent chromium in the medium was determined by diphenylcarbazide method (18). Total chromium was assayed by AA spectrophotometer (2380, Perkin Elmer, USA).

Results and Discussion

Isolation and identification of Cr⁺⁶ tolerating bacterium

The results of Cr⁺⁶ tolerant strains given in Table 1 show that 450 colonies appeared on basal medium containing 250 ppm Cr⁺⁶. These isolates were then transferred to the medium of higher concentrations of Cr⁺⁶. Only one bacterial strain, *C-171*, appeared on medium having 2000 ppm Cr⁺⁶. Hexavalent chromium is known to be toxic for various bacteria, e.g., *Bacillus subtilis*, *Escherichia coli*, and *Proteus vulgaris*, at low concentrations (19).

Table 1. No. of Cr⁺⁶ tolerant strain's colonies on agar plates.

S. no.	Concentration of Cr ⁺⁶ (ppm)	No. of tolerant strain's colonies
1	250	450
2	500	165
3	1000	20
4	1500	5
5	2000	1

The bacterium *C-171* was rod shaped and gram negative, of size 0.55 × 1.52 μ and motile. The cultural and physiological characteristics, given in Table 2, show that the bacterium belongs to the genus *Pseudomonas* and is named *Pseudomonas* sp. *C-171*.

Bacterial resistance to Cr⁺⁶ has been reported in *Pseudomonas ambigua* (20), *P. fluorescens* (21), *P. auriginosa* (22), *Aeromonas dechromaticans* (23), *Enterobacter cloacae* (11), and *Desulfovibrio* sp. (24). Nucleotide sequence analysis of *P. ambigua* by Cervantes et al. (22) revealed that a single open reading frame was sufficient to determine the resistance to Cr⁺⁶.

Resistance of the organism to Cr⁺⁶

The resistance of the isolated strain (*Pseudomonas* sp. *C-171*) towards the bactericidal action of Cr⁺⁶ was studied by using 2 types of chromium compounds, i.e. CrCl₃ (trivalent, pH 5.0) and K₂Cr₂O₇ (hexavalent, pH 7.0). The CrCl₃ has no bactericidal effect while K₂Cr₂O₇ exerted a powerful killing effect and killed more than 50% of cells in 20 min (Figure 1). The growth curves of *Pseudomonas* sp. *C-171* at different concentrations of Cr⁺⁶ as K₂Cr₂O₇ revealed that the rate of growth decreased with the increase in Cr⁺⁶ (Figure 2). When *Pseudomonas* sp. *C-171* was grown in medium above

Table 2. Characteristics of Cr⁺⁶ tolerating bacterium.

1. Morphological characteristics	
a. Shape and size:	Rod with rounded ends, 0.55 × 1.52 μ
b. Motility:	Motile
c. Gram reaction:	Negative
d. Growth:	Aerobic
2. Cultural Characteristics	
a. Nutrient agar colonies:	Circular, Smooth Convex, Translucent, Pigment produced
b. Slant culture:	Abundant, spreading growth
c. Stab culture:	Filiform
3. Physiological Characteristics	
a. Gelatin liquification	Positive
b. Indol	Negative
c. H ₂ S Production	Positive
d. Starch Hydrolysis	Negative
e. Catalase	Positive
f. Urease	Negative
g. Methyl red test	Negative
h. Voges-Proskauer reaction	Positive

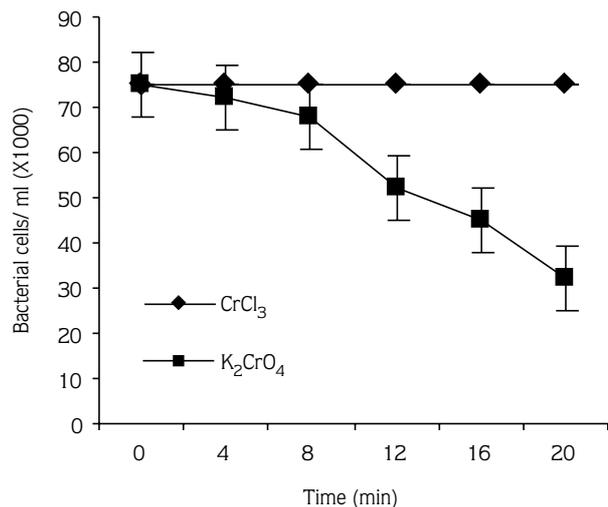


Figure 1. Killing curve of *Pseudomonas* sp. C-171 in different chromium compounds.

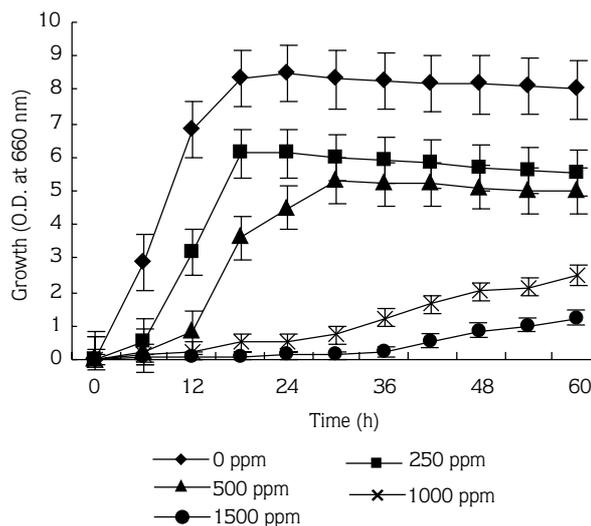


Figure 2. Growth curves of *Pseudomonas* sp. C-171 at various concentrations of Cr⁺⁶.

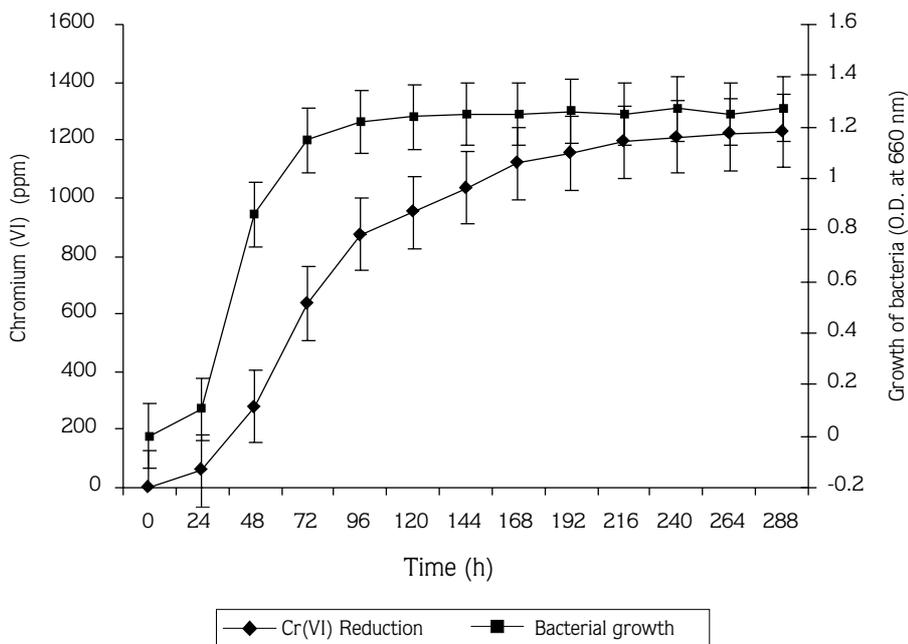


Figure 3. Reduction of Hexavalent chromium by *Pseudomonas* sp. C-171.

250 ppm Cr⁺⁶, the accumulation of precipitates of chromium hydroxide (Cr(OH)₃) around the bacterial cells and slight elongation of bacterial cells were observed. The accumulation of chromium hydroxide around the cells may have a protective effect against Cr⁺⁶ by limiting the contact of Cr⁺⁶ with the bacterial cells and consequently increase the tolerance of the bacterium towards toxicity.

Shimada and Matsushima (19) observed the elongation of cells above 1000 ppm K₂CrO₄ concentration.

Growth of *Pseudomonas* sp. C-171 and Reduction of Cr⁺⁶

Figure 3 shows the growth of *Pseudomonas* sp. C-171 and course of the Cr⁺⁶ reduction. Initially the rate of

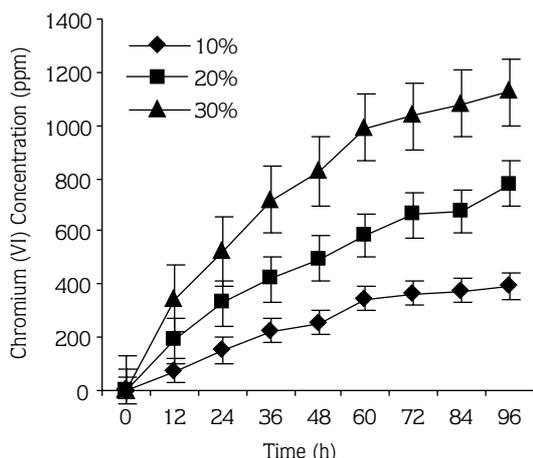


Figure 4. Effect of inoculum concentration on reduction of Cr^{+6} by *Pseudomonas sp. C-171*.

growth was slow due to the killing effect of Cr^{+6} , which was subdued with the passage of time. The accumulation of $\text{Cr}(\text{OH})_3$ around the bacterial cells probably protected them from the bactericidal effect of Cr^{+6} . The rate of Cr^{+6} reduction was slower during the lag (0 to 24 h) and stationary phase (96 to 288 h) than in the log phase (24 to 96 h). The rate of Cr^{+6} reduction during the log phase of *Pseudomonas sp. C-171* was because of the faster growth and maximum production of H_2S gas. This figure also revealed that the reduction of Cr^{+6} is directly related to the production of H_2S . The accumulation of $\text{Cr}(\text{OH})_3$ around the bacterial cells during the stationary phase of bacterial growth decreased the availability of H_2S for reaction with chromium, which consequently decreased/stabilized the rate of Cr^{+6} reduction.

References

- Ohtake H, Fuji E, Toda K. Bacterial reduction of hexavalent chromium: Kinetic aspects of chromate reduction by *Enterobacter cloacae* Ho 1. *Biotransformation* 4: 227-235, 1990.
- Chiong M, Gozalez E, Barra R et al. Purification and biochemical characterization of tellurite-reducing activities in *Pseudomonas thermophilus* HB8. *J Bacteriol* 170: 3269-3273, 1988.
- Lebedeva EV, Lyalikova NN. Reduction of crocoite by *Pseudomonas chromatophyllia sp. Nov.* *Mikrobiologiya* 48: 517-522, 1979.
- Summers AO, Silver S. Microbial transformations of metals. *Annu Rev Microbiol* 32: 637-672, 1978.
- Mayers CR, Neelson KH. Bacterial manganese reduction, growth with manganese oxide as the sole electron acceptor, *Science* 240: 1319-1321, 1988.
- Williams JW, Silver S. Bacterial resistance and detoxification of heavy metals. *Enzyme Microb Technol* 6: 530-537, 1984.
- Gerrard TL, Telford JN, Williams HH. Detection of selenium deposits in *Escherichia coli* by electron microscopy. *J Bacteriol* 119: 1057-1060, 1974.
- Smith WL. Hexavalent chromium reduction and precipitation by sulphate-reducing bacterial biofilms. *Environ Geochem Health*, 23: 297-300, 2001.

Effect of inoculum concentration on reduction of Cr^{+6}

The results depicted in Figure 4 show that the rate of Cr^{+6} reduction was dependent upon the concentration of inoculum. The increase in the inoculum concentration increased the reduction of Cr^{+6} . Maximum reduction of 1125 ppm was recorded with a 30% V/V inoculum concentration. This reduction was \approx 4-fold greater than with 10% and \approx 1.50-fold greater than with 20% inoculum. As the *Pseudomonas sp. C-171* is H_2S positive and produces H_2S during its growth, the increase in the inoculum size provided a higher number of bacterial cells, which in turn increased the rate of H_2S production, thus quickening the reduction of Cr^{+6} . The H_2S produced by the bacterium reacts with chromium to form chromium sulfide, which is not stable in aqueous solution and is rapidly deposited in the form of $\text{Cr}(\text{OH})_3$. Smillie et al. (25) reported reduction of $\text{Cr}(\text{VI})$ by bacterially produced H_2S in a marine environment. Fude et al. (15) also confirmed the mechanism of reduction of $\text{Cr}(\text{VI})$ through bacterially generated H_2S . The increase in inoculum size also increased the initial rate of Cr^{+6} reduction. The dependence of Cr^{+6} reductions on the cell density has also been reported by Wang et al. (12).

Corresponding author:

Mujeeb UR RAHMAN
 PCSIR Laboratories, P.O. Box 387,
 Mastung Road, Quetta,
 Balochistan, PAKISTAN
 E-mail: mujeeb_rn@hotmail.com

9. Shakoory AR, Makhdoom M. Hexavalent chromium reduction by a dichromate resistant gram-positive bacterium isolated from effluents of tanneries. *Appl Microbiol Biotechnol* 53: 348-351, 2000.
10. Schmieman EA, Yonge DR, Petersen JN et al. Kinetics of chromium reduction by mixed cultures during growth phase. *Water Environ Res* 72: 523-529, 2000.
11. Rahman M, Gul S, Din Z et al. Removal of chromium by a consortium of bacteria isolated from domestic sewage. *Pak J Biol Sci* 3: 2159-2162, 2000.
12. Wang PC, Mori T, Komori K et al. Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions. *Appl Environ Microbiol* 55: 1665-1669, 1989.
13. Kvasuikova EI, Stepanyuk VV, Klyushnidova TM et al. A new chromium reducing, gram variable bacterium with mixed type of flagellation. *Mikrobiologiya* 54: 83-88, 1984.
14. Ishibishi Y, Cervantes CK, Silver S. Chromium reduction in *Pseudomonas putida*. *Appl Environ Microbiol* 56: 2268-2270, 1990.
15. Fude L, Shigui L. Microbial removal and recovery of chromium(VI) from electroplating waste water. *J Sichuan Univ Nat Sci Ed* 2: 266-273, 1992.
16. Wang PC, Mori T, Komori K et al. Membrane associated chromate reductase activity from *Enterobacter cloacae*. *J Bacteriol* 172: 1670-1672, 1990.
17. Buchanan RE, Gibbons NE. *Bergey's Manual of Determinative Bacteriology*, 8th ed., Williams and Wilkins Co., Baltimore, 1974.
18. Urone PF. Stability of colorimetric reagent for chromium. S-diphenylcarbazides in various solvents. *Anal Chem* 27: 1354-1355, 1955.
19. Shimada K, Matsushima K. Isolation of potassium chromate resistant bacterium and reduction of hexavalent chromium by the bacterium. *Appl Environ Microbiol* 67: 101-106, 1983.
20. Horitsu H, Futo S, Ozawa K et al. Comparison of characteristics of hexavalent chromium tolerant bacterium *Pseudomonas ambigua* G-1 and its hexavalent chromium sensitive mutant. *Agric Biol Chem* 47: 2907-2908, 1983.
21. Bopp LH, Chakarabarty AM, Erlich HL. Chromate resistance plasmid in *Pseudomonas fluorescens*. *J Bacteriol* 155: 1105-1109, 1983.
22. Cervantes C, Ohtake H, Chu L et al. Cloning, nucleotide sequence and expression of the chromate resistance determinant of *Pseudomonas aeruginosa* plasmid pUM505. *J Bacteriol* 172: 287-291, 1988.
23. Serpokrylov NS, Zhukov IM, Simonova GA et al. Biological removal of chromium(VI) compounds from waste water by anaerobic microorganisms in the presence of organic substrate. USSR SU Patent, 119802, 1985.
24. Fude L, Harris B, Urrutia MM et al. Reduction of Cr(VI) by a consortium of sulphate reducing bacteria (SRB III). *Appl Environ Microbiol* 60: 1525-1531, 1994.
25. Smillie RH, Hunter K, Loutit M. Reduction of chromium (VI) by bacterially produced hydrogen sulphide in a marine environment. *Water Res* 15: 1351-1354, 1981.