Bull. Environ. Contam. Toxicol. (2005) 75:400–406

DOI: 10.1007/s00128-005-0767-z

Bulletin of Environmental Contamination and Toxicology

Chromate Reduction by *Serratia marcescens* Isolated From Tannery Effluent

V. L. Campos,[1] R. Moraga,[1] J. Yánez,[2] C. A. Zaror,[3] M. A. Mondaca[1]

[1] Microbiology Department, University of Concepción, Post Office Box 160-C, Correo 3, Concepción, Chile
[2] Chemical Inorganic and Analytical Department, University of Concepción, Post Office Box 160-C, Correo 3, Concepción, Chile
[3] Engineering Department, University of Concepción, Post Office Box 53-C, Correo 3, Concepción, Chile

Received: 19 November 2004/Accepted: 12 June 2005

Chromate compounds have many industrial applications and often cause environmental pollution in marine and freshwater sediments (Camargo et al. 2003). Hexavalent chromium (chromate, Cr(VI)), enters the environment as a result of effluent discharges from leather tanning, photografic-film making, car manufacturing. (Nkhalambayausi et al. 2001).

Cr(VI) is extremely toxic being carcinogenic and teratogenic (Vonburg and Liu 1993). Trivalent chromium is relatively unreactive, less toxic than Cr(VI), and may be precipitated out as phosphate in neutral solution (Barlett and James 1988).

The presence of chromate in the environment inhibits most bacteria (Cervantes 1990). On the other hand, some species could tolerate the presence of chromate, as also degrade those pollutants (Rore et al. 1994). Indeed, bacterial chromate resistance is commonly conferred by plasmids (Cervantes and Silver 1992). Cr(VI) reduction and Cr(VI) resistance have been considered to be unrelated (Ohtake et al. 1987). There are many examples where chromate resistance is due to the presence of efflux mechanisms which allow resistant strains to extrude CrO_4^{-2} ions. Thus, low CrO_4^{-2} uptake of was found in *Pseudomonas fluorescens* LB300 (Bopp and Ehrlich 1988), *P. Aeruginosa* (Cervantes and Ohtake 1988) *Alcaligenes eutrophus* (Nies and Silver 1989), and *E. cloacae* (Ohtake et al. 1990).

Many microorganisms have been reported to reduce the highly soluble and toxic hexavalent chromium (chromate: Cr(VI)) to the less soluble and less toxic Cr(III), e.g. *Streptomyces* sp. (Das and Chandra 1990), *Pseudomonas putida* (Ishibashi et al. 1990), *Agrobacterium radiobacter* (Llovera S et al. 1993), *Desulfovibrio vulgaris* (Lovley and Phillips 1994), *Bacillus* sp (Campos et al. 1995), *Microbacterium* sp. MP30 (Pattanapipitpaisal et al. 2001).

Aerobic chromate reductase activity is generally associated with a soluble protein fraction utilizing NADH as an electron donor (Ishibashi et al. 1990), whereas anaerobic chromate reduction is reported to occur at the membrane (Wang et al. 1991). Thus, reduction of Cr (VI) to Cr (III) by bacteria might be a potential treatment for remediating chromate contaminated waters and soils (Lovley 1993).

Correspondence to: M. A. Mondaca

Within such context, this work reports on experimental results obtained from *Serratia marcescens*, isolated from tannery effluent, which demonstrate Cr(VI) reduction activity.

MATERIALS AND METHODS

Serratia marcescens, chromate-resistant bacteria, was isolated from a local tannery effluent by attachment to an artificial epilithon. Sterile porous stones were submerged in a tannery effluent channel a period of 7 days, to ensure the formation of a superficial epilithon. Stones were then taken to the laboratory where the epilithic suspension was obtained (Bale et al. 1988). Samples from this suspension were cultivated in Plate Count Agar (Merck) containing 0.5 mM potassium chromate (Merck, p.a.), at 30°C for 48 h. The bacteria was identified using a rapid identification kit (EM-Ident E, Sensildent, Merck Darmsdat, Germany). Microtitration plates were inoculated with 0.1 ml of bacterial suspension (10^5-10^6 CFU/ml). Identification with EM-Ident-E combines 21 biochemical reactions, which are evaluated and interpreted after incubation for 18-24 h at 37°C (Curtis et al. 1970).

The strain was cultured in KSC medium with chromate (Cr (VI)) 0.5 mM, at 30°C for 24 h under aerobic conditions (Ishibashi et al. 1990). Bacterial growth was measured by turbidimetry at 660 nm using a spectrophotometer Cecil 3000. (Wang et al. 1989) and also by viable counts on a Plate count agar (PCA).

Preliminary chromate reduction was determined colorimetrically with a spectrophotometer Cecil 3000 at 540 nm by reaction with diphenylcarbazide in acid solution (American Public Health Association 1971). Analyses of Cr(VI) and Cr(III) were also performated using High Pressure Liquid Chromatography (Merck-Hitachi, L-100 model) coupled on-line with a Flame Absorption Atomic Spectrometer (Perkin Elmer, Analyst 100 model) (HPLC-FAAS) for simultaneus separation and determination of both chemical species. Samples were withdrawn at different times and centrifuged at 3000 rpm for 5 min prior to analysis.

Metal precipitates by energy-dispersive X-ray spectroscopy (EDS), using uncontrasted sections were observed and characterized. Samples of cells loaded with chromate were washed with isotonic saline and then with distilled water. The pellet was resuspended in distilled water to prepare a suspension. One drop of this suspension was placed on a copper electron microscope grid, pre-coated with a carbon film, and air-dried overnight. These grids were examined by transmission electron microscopy JEOL, model JEM-1200EX II (Macaskie et al. 1987).

RESULTS AND DISCUSSION

The main characteristics of isolated strain are shown in Table 1. On the basis of morphological and biochemical properties, this bacterium was identified as *Serratia marcescens* at a confidence level of 91.7%.

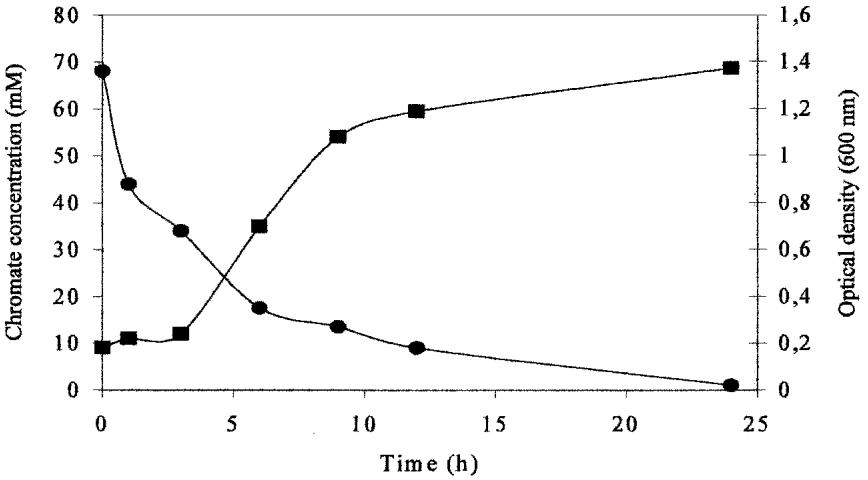

Figure 1. Batch bacterial growth in KSC medium with 0.5 mM chromate. The chromate reduction was determined colorimetrically by reaction with diphenylcarbazide in acid solution. (■)Chromate reduction. (●) Bacterial growth.

The effect of chromate on of *S. marcescens* growth was tested under aerobic conditions. The strain could grow without any inhibition at a chromate concentration of 2 mM. It must be emphasized that resistance to chromate is not a common property of *Serratia spp.*, since normal enterobacteria cannot tolerate the presence of chromate at those levels. Indeed, strains of *Escherichia coli*, *S. marcescens* and *Enterobacter aerogenes* could not grow in the presence of 1 mM chromate (Wang et al. 1989). *S. marcescens* cells were grown aerobically in KSC medium containing 0.5 mM chromate (Cr(VI)). As seen in figure 1, turbidity increased as chromate concentrations decreased. Cell-free KSC medium did not show any reduction in chromate concentration. Aerobic incubation of *S. marcescens* cultures mixture with 0.5 mM chromate (Cr(VI)) caused a decrease of 0.5 mM chromate in 24 h, such decrease in chromate concentration could be accounted for by biological reduce to Cr(III).

Indeed, the reduction of Cr(VI) to Cr(III) was verified using HPLC-FAAS (Posta et al. 1993). As seen in figure 2, Cr(VI) concentrations decreased 86%, with the consequent appearance of Cr(III), after 48 h incubation. A mass balance shows that most Cr(VI) was transformed to he less toxic and relatively insoluble Cr(III) at a rate of 1.51 mg l^{-1} h^{-1}. Although this strain was able to both reduce and tolerate chromate, these two properties may be unrelated. Indeed, it is not clear whether the chromate reduction ability found with several bacterial isolates confers resistance to chromate (Ohtake et al. 1987). The ability to reduce chromate may be a secondary activity of a soluble reductase enzyme with a quite different physiological role (Ishibashi et al. 1990; Shen and Wang 1993).

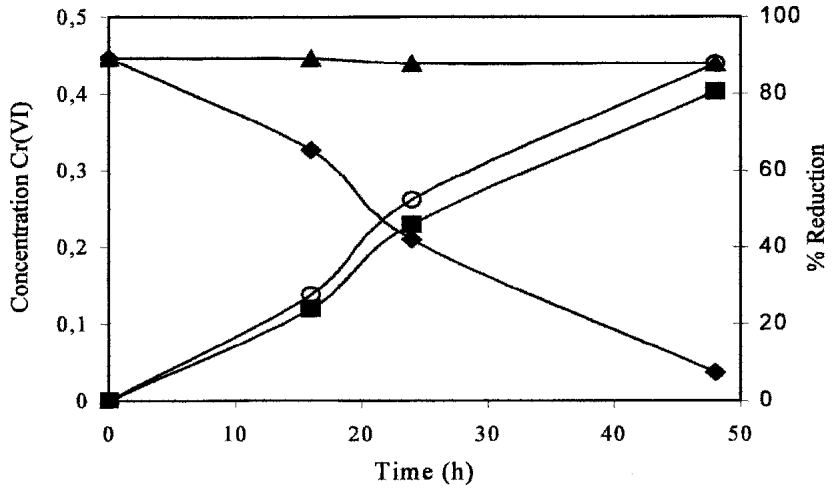

Figure 2. Batch bacterial culture in KSC medium, conversion of Cr(VI) to Cr(III). Cr(VI) and Cr(III) were determined by HPLC-FAAS. (♦) Cr((VI), (■) Cr(III), (▲) Mass balance, (○) % reduction of Cr(VI).

Transmission electron microscopic examination of *S. marcescens* cells revealed that electron scattering particles were deposited on the outside of bacterial cells. Moreover, as seen in Figure 3, energy-dispersive X-ray analyses showed that these precipitates contained chromium. These observations show that insoluble chromium was mainly accumulated outside cells. Similar results for *Pseudomonas sp.* were reported by Wang et al. (1990) indicating that reductase acts extracellularly. Previous work had shown that when Cr(VI) was reduced to Cr(III), intracellular Cr(III) cannot be removed from cells as long as their membrane remained intact (Aaseth et al. 1982).Others have also proposed that microbial Cr(VI) reduction occurs most likely on the cell surface (Wang et al. 1990), although minor intracellular reduction of Cr(VI) may also take place. The formation of insoluble Cr(III) on the cell surface was thought to offer protection against Cr(VI) toxicity (Wang et al. 1990; Shen and Wang 1993).

This is the first published report on chromate reductase activity of *Serratia marcescens* and results showed that Cr(VI) was quantitatively converted to Cr(III), which mainly existed in the external surface of *S. marcescens* cells. These characteristics of resistence to chromate and reduction activity identify *S. marcescens* as a suitable bacteria to detoxify chromate from chromium-containing industrial discharges. Chromate biological reduction is an excellent candidate technology for low-cost bioremediation, especially in developing countries, where leather tanneries and other chromate-consuming processes pose environmental concerns.

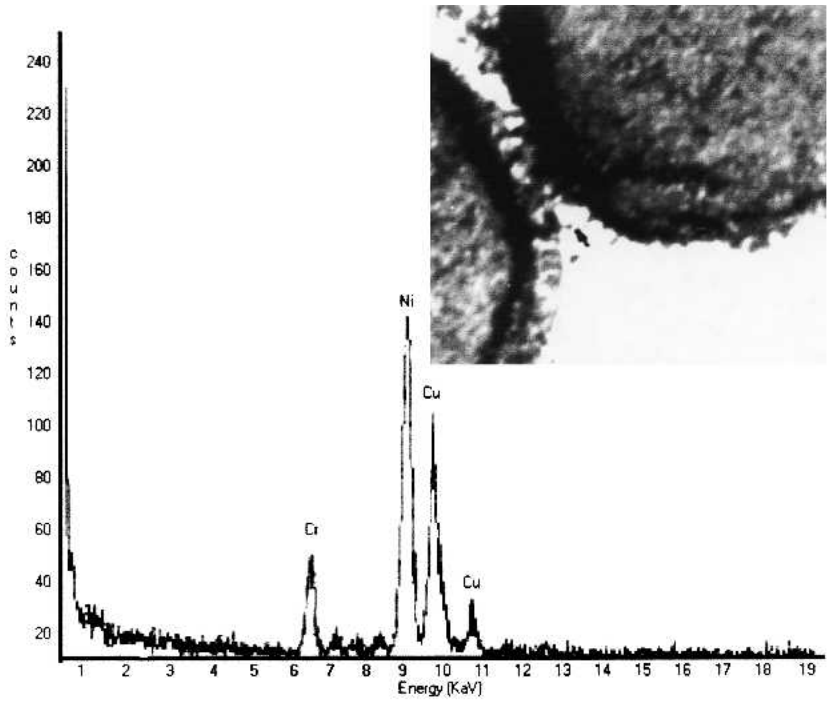

Figure 3. Transmission electron microscopy, with X-ray diffraction analysis, of cultured bacterial cells, showing the presence of $Cr(OH)_3$ precipitated around cells.

Table 1. Biochemical characteristics of *S. marcescens* strain

Test		Test	
Trytophan-de/s/aminase	-	PGUR (p-nitro-β-glucuronide)	-
Esculin	+	H_2S	-
Indole	-	Harnstoff/urea	-
Lysine-decarboxylase	+	Ornithine-decarboxylase	-
Arginine-dehydrolase	-	Glucose / control for sugar	+
Citrate	-	Malonate	-
Voges-Proskauer	+	Saccharose/sucrose	+
Rhamnose	-	Inositol	+
Adonitol	+	Sorbitol	+
Xylose	+	O-nitrophenyl-xylopyranoside	-
O-nitrophenyl-β-galactopyranoside	+		

Acknowledgments. We thank Mr. R. Alarcón of the Dirección de Investigación of Universidad de Concepción, for their technical assistance. This work was financially supported by DIUC 99.036.016-1.0, to whom the authors are greateful.

REFERENCES

Aaseth J, Alexander J and Norseth T (1982) Uptake of 51Cr-chromate by human erythrocytes a role of ghitathione. Acta Pharmacol Toxicol 50:310-315.

American Public Health Association. (1971) Chromium, pp 115-159. In: Standard Methods for the Examination of Water and Wastewater. American Public Health Association, New York.

Bale M, Fry JC and Day MJ (1988) Transfer and occurrence of large mercury resistance plasmid in river epilithon. Appl Environ Microbiol 54: 972- 978.

Barlett R and James B (1988) Mobility and bioavailability of chromium in soils. In Chromium in The Natural and Human Environments ed. Nriagu, J. And Nieboer, E. pp. 267-383. New York: Wiley and Sons.

Bopp L H and Ehrlich H (1988) Chromate resistance and reduction in *Pseudomonas fluorescens* strain LB 300. Arch Microbiol 150: 426-431.

Camargo FA, Bento FM, Okeke BC, Frankenberger WT. 2003. Chromate reduction by chromium-resistant bacteria isolated from soils contaminated with dichromate. J Environ Qual. 32:1228-33.

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

Cervantes C (1990) Bacterial resistence to copper. Rev Latin Microbiol 32: 321-325.

Cervantes C and Ohtake H (1988) Plasmid-determined resistance to chromate in *Pseudomonas aeruginosa.* FEMS Microbiol Lett 56:173–176.

Cervantes C and Silver S (1992) Plasmid chromate resistence and chromate reduction. Plasmid 27:65-71.

Curtis M.A, Lapage SP, Bascomp S and Wilcox WR (1970) Computer identification of Gram-negative rods, in: Automation, Mechanization and Data Handling in Microbiology. Society for Applied Bacteriology Technology. Ser. N° 4, Baillie, A.M. and Gilbert R.J., Academic Press, London.

Das S and Chandra AL (1990) Chromate reduction in *Streptomyces*. Experientia 46:731-733.

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

Llovera S, Bonet R, Simon-Pujol MD and Congregado F (1993) Chromate reduction by resting cells of Agrobacterium radiobacter EPS-916. Appl Environ Microbiol 55: 1665-1669.

Lovley DR (1993) Dissimilatory metal reduction. Ann Rev Microbiol 47:263-290.

Lovley DR and Phillips E (1994) Reduction of chromate by *Desulfovibrio vulgaris* and its C3 cytochrome. Appl Environ Microbiol 60: 726-728.

Macaskie LE, Dean A, Cheethons AK, Jakeman R and Sharmulis AJ (1987) The chemical nature of the accumulated metal precipitate and its location on the bacterial cells. J Gen Microbiol 133: 539-544.

Nies A and Silver S (1989) Plasmid-determined inducible efflux is responsible for resistance to cadmium, zinc and cobalt in Alcaligenes eutrophus. J Bacteriol 171: 896–900

Nkhalambayausi-Chirwa EM, Wang YT. 2001. Simultaneous chromium(VI) reduction and phenol degradation in a fixed-film coculture bioreactor: reactor performance. Water Res 35: 1921-32.

Ohtake H, Cervantes C and Silver S (1987) Decreased chromate uptake in *Pseudomonas fluorescens* carryng a chromate resistence plasmid. J Bacteriol 169: 3853-3856.

Ohtake H, Fujii E, Toda K (1990) Reduction of toxic chromate in an industrial effluent by use of a chromate-reducing strain *Enterobacter cloacae*. Environ Technol 11: 663–668

Pattanapipitpaisal P, Brown N and Macaskie L (2001) Chromate reduction and 16s rRNA identification of bacteria isolated from a Cr(VI)-contaminated site. Appl Microbiol Biotechnol 57: 257-261.

Posta J, Berndt H, Lou S and Schaldach G (1993) High-Performace flow flame atomic absorption spectrometry for automated on line separation and determination of Cr(III)/Cr(VI) and preconcentration of Cr(VI). Anal Chem 65: 2590-2595.

Rore D, Top F, Houwen F, Mergeay M and Verstraete W 1994. evolution of heavy metal resistant transjugants in soil environment with a concomintant selective pressure, FEMS Microbiol Ecol 14: 263-274.

Shen H and Wang Y (1993) Characterization of enzymatic reduction of hexavalent chromiun by *Escherichia coli* ATCC 33456. Appl Environ Microbiol 59: 3771-3777.

Vonburg R and Liu D (1993) Chromium and hexavalent chromium. J Appl Toxicol 13: 225-230.

Wang P, Mori T, Komori K, Sasatsu M, Toda K and Ohtake H (1989) Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions. Appl Environ Microbiol 55: 1665-1669.

Wang P, Toda K, Ohtake H, Kusaka Y and Yabe Y (1991) Membrane-bound respiratory system of *Enterobacter cloacae* strain HO1 grown anaerobically with chromate. FEMS Microbiol Lett. 78: 11-16.

Wang, P, Mori T, Toda K and Ohtake H (1990) Membrane-associated chromate reductase activity from *Enterobacter cloacae*. J Bacteriol 172: 1670-1672.