# Chapter 5
# Role of Bacterial Chromate Reductase in Bioremediation of Chromium-Containing Wastes

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## ABSTRACT

*Chromium toxicity is a major environmental concern as it is the chief environmental pollutant released by paint, stainless steel, and mining industries. In nature, chromium exists in two stable valance states: Cr(VI) and Cr(III). Cr(VI) is highly toxic and soluble at neutral pH, whereas Cr(III) is insoluble at normal pH and is less toxic. Thus, it is essential to draw strategies for mitigation of Cr(VI), and microbial reduction of toxic Cr(VI) has been identified as a bioremediation technique not only to detoxify chromium but also to recover the non-toxic Cr(III) by physical means. Chromate reductase, the central enzyme involved in bioreduction of Cr(VI) to Cr(III) may be both intracellular as well as extracellular in nature. Most of the chromate reductase enzyme belongs to the oxidoreductase group such as nitroreductase, iron reductase, quinone reductase, hydrogenase, flavin reductase, as well as NAD(P)H-dependent reductase. Detailed analysis of the structure of the enzymes will help us in the suitable application of these enzymes in bioremediation of metal-contaminated wastes.*

## INTRODUCTION

Contamination of heavy metal is considered as a serious environmental problem causing serious health hazards all over the world. They are released mainly due to anthropogenic activities, mineral processing and mining activities and their release has increased enormously in the past few decades. Their mitigation has become very necessary as well as a challenging task for mankind and it has received a lot of attention. Consequently more strict legislation for the protection of the environment has gradually become indispensible to reduce the release of heavy metal containing waste in the water bodies.

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Chromium is one of the frequently used metal and is released from different industrial sources such as electroplating, stainless steel, dye and leather industries. It is emerging as one of the top 20 contaminants and is considered as a hazardous substance for the past 15 years (United States Environmental Protection Agency, 1998; Ryan et al. 2002). In India a huge amount of elemental Cr are released annually through waste water by different industries and concentration of Cr in these water ranges between 2 to 5 g/L much higher than the maximum permissible limit (2 mg/L).

Chromium mainly exists in nature in different valence states ranging from -4 to +6, with the hexavalent species and trivalent species considered as the most stable state. Cr(VI) is predominant in natural aquifers and mobile, can penetrate the biological membrane and is considered as mutagenic, carcinogenic and teratogenic in nature. Hexavalent chromium mostly exists as chromate ions ($CrO^4$) which has structural similarities with sulphate ions ($SO^4$) and therefore can enter the cell via the sulphate transport system present in the cell membrane. Once inside the cell chromate ion being an oxyanion readily forms DNA adducts and causes mutations. On the other hand trivalent counterpart Cr(III) prevails in the municipal wastewater rich in organic substances (Fukai, 1967; Jan and Young, 1978), it is thermostable in nature, less mobile and is therefore much less harmful.

Several chemical treatments such as chemical reduction followed by precipitation, and adsorption and processes such as reverse osmosis, electrodialysis and ion exchange processes are used to remove and reduce hexavalent chromium and precipitate them. However, they are expensive with high energy and chemical consumption, which also generates a huge amount of toxic sludge and secondary wastes. Moreover, most of these treatments are applicable if the concentration of chromium ranges between 1 to 100 mg/L. So the use of bioremediation technology is nowadays considered as a more viable option in removal of Cr(VI) from waste waters and contaminated lands. Several bacteria exists which in both living as well as non living condition have the capacity to reduce, accumulate and absorb Cr(VI) ions. Several bacterial strains isolated from different environment are reported to be capable of reducing toxic and mutagenic Cr(VI) to less toxic Cr(III) as well as precipitate it at neutral pH. The enzyme playing a central role in the reduction process is the chromate reductase which is either NADH or NADPH dependent. Mostly these enzymes belong to the hydrolases, dehalogenases, transferases and oxidoreductases class and can either be intracellular or extracellular in nature. Most of the chromium reductases reported till now works in aerobic conditions and is normally associated with soluble protein fractions and the reduction process is carried out either internal or external to the plasma membrane. Whereas, those chromate reductase which works under anaerobic condition, uses Cr(VI) as terminal electron acceptor and is membrane bound.

The present study mainly focuses on the production, mode of action and application of these enzymes under both free and immobilized conditions for bioremediation of hexavalent chromium.

## CHROMIUM TOXICITY

As described in the previous section chromium is released from different sources such as industrial effluent and mining effluent which contaminates the soils and water bodies. Chromium exists in nature in different valance form, however, hexavalent and trivalent states are found to be most stable in nature. Hexavalent chromium can also be generated from trivalent chromium due to environmental oxidation. Cr(VI) is highly toxic and can penetrate the cell membrane through the sulphate transporter pathway. When within the cell they are reduced to the trivalent species in presence of ascorbate and glutathione