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Review

Bacterial chromate reductase, a potential enzyme for bioremediation of hexavalent chromium: A review

Hrudayanath Thatoi [a, *], Sasmita Das [a], Jigni Mishra [a], Bhagwat Prasad Rath [a], Nigamananda Das [b]

[a] Department of Biotechnology, College of Engineering and Technology, Biju Patnaik University of Technology, Techno-Campus, Ghatikia, Bhubaneswar 751003, Odisha, India
[b] Department of Chemistry, North Orissa University, Takatpur, Baripada 757003, Odisha, India

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ABSTRACT

Hexavalent chromium is mobile, highly toxic and considered as a priority environmental pollutant. Chromate reductases, found in chromium resistant bacteria are known to catalyse the reduction of Cr(VI) to Cr(III) and have recently received particular attention for their potential use in bioremediation process. Different chromate reductases such as ChrR, YieF, NemA and LpDH, have been identified from bacterial sources which are located either in soluble fractions (cytoplasm) or bound to the membrane of the bacterial cell. The reducing conditions under which these enzymes are functional can either be aerobic or anaerobic or sometimes both. Enzymatic reduction of Cr(VI) to Cr(III) involves transfer of electrons from electron donors like NAD(P)H to Cr(VI) and simultaneous generation of reactive oxygen species (ROS). Based on the steps involved in electron transfer to Cr(VI) and the subsequent amount of ROS generated, two reaction mechanisms, namely, Class I "tight" and Class II "semi tight" have been proposed. The present review discusses on the types of chromate reductases found in different bacteria, their mode of action and potential applications in bioremediation of hexavalent chromium both under free and immobilize conditions. Besides, techniques used in characterization of the Cr (VI) reduced products were also discussed.

1. Introduction

Environmental pollution due to indiscriminate discharge of hazardous and harmful wastes containing toxic heavy metals at elevated concentrations from industries and mining sites has been a growing concern all over the world and therefore, underline the importance of applying effective treatment methods to reduce the concentration of heavy metals down to acceptable limit. Among various approaches, bioremediation using biological agents such as bacteria, fungi, and their enzyme is one of the attractive and effective methods for cleaning the environment from toxic pollutants (Ruggaber and Talley, 2006). The microorganisms play an important role in bioremediation processes which is, however, limited by several factors. For instance, the microorganisms that are actively involved in the bioremediation of a specific pollutant might be inhibited by other pollutants present in the same environment. Further, the rate of degradation of pollutants by microorganisms is often very slow which limits the feasibility of using them in practice for bioremediation processes (Whiteley and Lee, 2006). In this context, the use of sole enzymes isolated from bacterial species is more advantageous than using whole microorganisms as revealed from several studies undertaken during last few years (Sutherland et al., 2004; Pieper et al., 2004). Moreover, the enzymatic biotransformations do not generate toxic side products as often found in the case of chemical and some microbiological processes and therefore, possess less risk of biological contamination on ecosystem. Their action is specific to the substrate in comparison to microorganisms and they are also more mobile than microorganisms because of their smaller size (Gianfreda and Bollag, 2002).

Although the enzymatic treatment processes have tremendous scope for bioremediation, its practical application often faces with several challenges in terms low activity, productivity and stability of the enzyme in addition to sustainability of their application. Efforts are on in search of potential microbes capable of producing enzymes that can transform the toxic metal ions to their less/non-toxic forms under wide range of environmental conditions (e.g. pH,

* Corresponding author.
E-mail address: hn_thatoi@rediffmail.com (H. Thatoi).

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temperature, presence interring species etc.) for their practical application in bioremediation processes.

Traditionally, most of the enzymes used in bioremediation process are confined to bacterial mono- or di-oxygenases, reductases, dehalogenases, cytochrome P450 mono-oxygenases; enzymes involved in lignin metabolism (such as laccases, lignin and manganese peroxidases isolated from white-rot fungi) and bacterial phosphotriesterases (Pieper et al., 2004). The most representative class of enzyme used in the remediation of polluted environments are hydrolases, dehalogenases, transferases and oxidoreductases; mainly obtained from bacteria, fungi, plants and microbe–plant associations (Rao et al., 2010). These enzymes are either intracellular (produced but retained within the originating microbial cells) or, extracellular (exported outside from the originating microbial cells) in nature. Enzyme production by microorganisms is usually low in its natural conditions. The production can be enhanced, although to a limited extent, in laboratory conditions through optimization of growth parameters. On the otherhand, the recombinant DNA technology offers a cost effective process for large scale production of enzymes with enhanced stability and activity (Alcalde et al., 2006). In fact, the production of enzymes in industrial scale from the suitable microbial strain is of paramount importance for wide spread practical applications of enzymatic bioremediation process. Keeping the above in view, the present review highlights the production of chromate reductase enzymes from bacterial sources, their mode of action and prospects of their applications both under free and immobilized conditions for bioremediation of one of the toxic environmental pollutants *viz.* hexavalent chromium.

2. Chromium toxicity and bioremediation options

The widespread industrial uses of chromium or its compounds and mining activities result the release of Cr-containing wastes into the environment that contaminate the soils, sediment and surafe/ground waters. Although essential for numerous living organisms in trace quantities, Cr is toxic at elevated levels. As a transition mental, it exists in different valence states ranging from −II to +VI with Cr(VI) and Cr(III) being the dominant species in the environment. Out of two commonly occurring states, Cr(VI) is toxic to biological systems due to its strong oxidizing potential that invariably damage the cells (Kotas and Stasicka, 2000). Cr(VI) is known to harmful to all forms of living systems (Wise et al., 2004) including microorganisms (Ackerley et al., 2006). Moreover, it is mutagenic (Puzon et al., 2002), carcinogenic (Codd et al., 2003), teratogenic (Asmatullah et al., 1998), and has been classified as one of the priority pollutants by several regulatory agencies including United States Environmental Protection Agency (USEPA) that pose greatest threat to humans (Cheung and Gu, 2007). Hexavalent chromium usually enters the cell via sulphate transporter pathway and gets reduced to Cr(III) by various enzymatic and non-enzymatic processes. During this process, the reactive oxygen species (ROS) are formed that exert deleterious effects on cells by interacting with protein as well as nucleic acid (Cheung and Gu, 2007). In contrast, trivalent chromium is having much less toxicity and bioavailabilty (He et al., 2009) as it readily forms insoluble hydroxide/oxides above pH ~ 5.5. In fact, the biological cell membranes are nearly impermeable to Cr(III). As such, detoxification of Cr(VI) by its reduction to Cr(III) is of great environmental importance.

Chemical reduction of hexavalent chromium to trivalent form followed by precipitation is the most common and widely used methods among others (e.g. electrochemical treatment, reverse osmosis, adsorption and ion-exchange) employed for its removal from contaminated bodies. It involves the reduction of Cr(VI) by many reducing agents such as Fe(0), Fe(II), sulphide, organic C-based materials etc. Chemical methods usually require high energy inputs and/or large quantity of chemical reagent. Besides, these methods are ineffective at lower concentration of Cr(VI) present in large volume of wastewaters and generate large quantity of toxic sludges, disposal of which again causes secondary pollution. On the other hand, biological methods such as microbial detoxification of Cr(VI) are economical, safe, and sustainable (Shakoori et al., 2000; Eccles, 1995) and also free from residual pollution problems. Many bacterial species possess chromate reductase activity, where the enzyme converts the highly toxic and soluble hexavalent chromium to less toxic trivalent form having much lower solubility; thereby reduction by the enzymes affords a means of chromate bioremediation (Park et al., 2000). In recent years, chromate reductases have raised enormous interest among the researcher across the globe because of their central role in mediating chromium toxicity and their potential use in bioremediation/biocatalysis (Ackerley et al., 2004b). This results in isolation of diverse chromate reductase bacterial species, characterization and their use in reduction of Cr(VI) to Cr(III) to develop a relatively environment friendly process alternative to the conventional methods.

3. Bacterial resistance to Cr(VI)

The chromosomal resistance in bacteria makes use of strategies like specific or unspecific Cr(VI) reduction, free radical detoxifying activities, repair of DNA damage (Morais et al., 2011) and processes associated with sulphur or iron homeostasis (Ramirez-Diaz et al., 2008). Many microorganisms have the potential to survive toxic metal-polluted environments by developing mechanisms to avoid metal toxicity like, metal efflux, adsorption uptake, DNA methylation, and metal biotransformation either directly by enzymatic reduction to less mobile and toxic forms or indirectly through making complexes with metabolites (such as H_2S) (Camargo et al., 2005; Pei et al., 2009; Soni et al., 2012). Microbial reduction of Cr(VI) to Cr(III) is particularly important from bioremediation point of view which can be considered as an additional chromate resistance mechanism (Cervantes et al., 2001). A variety of Cr-resistant bacteria with high Cr(VI)-reducing potential have been reported including *Pseudomonas*, *Bacillus*, *Enterobacter*, *Deinococcus*, *Shewanella*, *Agrobacterium*, *Escherichia*, *Thermus* and other species (Ohtake et al., 1987). It has been reported that both chromate resistant as well as non-resistant strains can reduce chromate but the growth of later are significantly inhibited at higher concentrations of chromate (Bopp and Ehrlich, 1988). Therefore, the bacterial property, which is particularly useful for an effective bioremediation approach, is one that combines high tolerance/resistance with the ability to reduce Cr(VI) to Cr(III) (Dhal et al., 2013).

Several microorganisms exhibiting Cr(VI)-reducing activities and resistance have been isolated and identified from chromate-contaminated environment as well as natural, uncontaminated ecosystems (Schmieman et al., 1998; Turick et al., 1996; Wang and Shen, 1995). Microorganisms that have the ability to reduce Cr(VI) are usually called as chromium reducing bacteria (CRB). Among CRB, the Gram-positive bacteria are shown to have significant tolerance to Cr(VI) toxicity at relatively high concentrations, whereas Gram-negative bacteria are much more sensitive to Cr(VI) (Coleman, 1988). Microorganisms found in metal contaminated environment are naturally resistant for such metals. An investigation carried out by Das et al. (2013) revealed that the bacteria isolated from chromite mine soils are resistant towards Cr(VI) along with other heavy metals. It is well known that chromate resistance and reduction are not necessarily interrelated, and not all Cr(VI)-resistant bacteria can reduce Cr(VI) to Cr(III). Thus, both chromium resistance and reduction are found to be independent