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REVIEW ON GENETIC REGULATION REDUCTION AND RESISTANT MECHANISM OF CHROMIUM

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Abstract
Chromium is a toxic heavy metal which is usually discharged from industrial waste water and also found in the soil of chromium contaminated area. Chromium is followed by a wide range of oxidation states where Cr(VI) and Cr(III) are mainly studied because it shows toxic, carcinogenic and mutagenic effects. Some microorganisms like Pseudomonas aeruginosa, E. coli, P. putida, S. oneidensis, Arthrobacter sp. Bacillus sp. etc. have the ability to reduce Cr(VI) and also shows resistant mechanism against Cr(VI). These bacteria follows efflux mechanism, sulfate transport mechanism, iron uptake mechanism, reduction by genes and proteins, reduction by EPS, reduction of chromium by extracellular, intracellular and membrane associated process techniques for their resistant mechanism. This review mainly gives an overview about the above mentioned mechanisms followed by bacteria to show the resistant capacity which can not only reduce environmental pollution but also protects human health.

Keywords: Chromium, Bacteria, Bioremediation, Resistant Mechanism.

1. Introduction

Heavy metals are known to be toxic in nature and use of these metals in high amount can be harmful to human because it inhibits the metabolic rate. Chromium is one of those metals which is remarkable for its magnetic properties, it is the only elemental solid which shows antiferromagnetic ordering at room temperature. Above 38 °C, it changes to paramagnetic. Maximum amount of chromium is discharged from industry like petroleum industry, iron industry, steel industry, metal finishing industry and leather tanning industry which effects the human health, aquatic lives, land, vegetables farming and crops etc. Hexavalent Chromium is one of a compound of chromium which is mainly studied because it is a toxic heavy metal with both carcinogenic and mutagenic effect. It also has many other effect like mental retardation, growth and development of abnormalities and wide range of other illness. Because Cr(VI) has water soluble and strong oxidizing in nature so that Cr(III) has found to be less toxic. Some studies reveal that by reducing Cr(VI) to Cr(III) some of the previously discussed problems can be solved. According to researchers some microorganisms has received a great attention in this process. Many bacteria are found to reduce Chromium (VI) to Chromium (III) under aerobic, anaerobic and also in both conditions. *Shewanella oneidensis* MR-1 is a facultatively anaerobic γ-proteo bacterium, which has the ability to transform Cr(VI) to Cr(III) (Myers *et al.* 2000; Viamajala *et al.* 2002). *Intrasporangium* sp. Q5-1, *Bacillus* sp. ES29, *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas fluorescens* LB300 are also reported to reduce Cr(VI) to Cr(III), under aerobic and anaerobic condition (Ilias *et. al.* 2011). Some intrinsic bacteria are also found which can reduce chromium. These microorganisms have diverse resistant mechanisms, these include biosorption, diminished intracellular accumulation through either direct obstruction of the ion uptake system or active chromate efflux, precipitation, and reduction of Cr(VI) to Cr(III). Some species of *Pseudomonas* (Bopp *et al.* 1983; Cervantes *et al.* 1988; Summers *et al.* 1978) and *Alcaligenes* (Nies *et al.* 1989) also have Plasmid-determined resistance mechanism to chromate. According to researchers in these microorganism there are some proteins responsible for chromium reduction. ChrA is a hydrophobic protein with 12 proposed transmembrane-spanning domains (Cervantes *et al.* 2001; Cervantes *et al.* 1990; Nies *et al.* 1990) found to be responsible for the plasmid-specified resistance phenotype in these organisms and function as a secondary transport system for the extrusion of chromium ions (Alvarez *et al.* 1999; Chourey *et al.* 2006). The soluble enzymes that is a group of cytoplasmic dimeric flavoprotein has chromate reductase activity in it and it also reduce Cr(VI)-Cr(III) (Park *et al.* 2000; Ackerley *et al.* 2004b). A flavin reductase (Fre) system from E. coli (Puzon *et al.* 2005) and *Thermus scotoductus* SA-01,(Opperman *et al.* 2007) isolated from a South African gold mine also shows chromium(VI) reduction activity. Many microorganisms have showed chromium resistant mechanism but researchers are nowadays studying for some naturally occurring bacteria which can accumulate heavy metals from the industrial sites and research are still going on both enzymatic as well as cellular levels to make bacteria efficient agents of chromate bioremediation.

2. Efflux Mechanism

In general, most of the bacterial species are not found to be Cr(VI) resistant but some of them contain a Cr(VI) reduction gene in their plasmid and chromosomes, which helps to remove chromium. This is followed by a mechanism called efflux process. Previous studies suggested that plasmid pUM505 and pMOL28 of *Pseudomonas aeruginosa* and *Alcaligenes*

eutrophus can reduce Cr(VI) and Cr(III) because of a protein called ChrA present in chrA gene of this plasmid. They also suggested that the membrane vesicle of Cr-resistant *Pseudomonas aeruginosa* which has ChrA protein, can accumulate four fold more chromium than plasmid less vesicles (Alvarez *et al.* 1999). *Pseudomonas aeruginosa* and *Cupriavidus metallidurans* are reported as model organism of chromate efflux system and can resist upto 0.3mM and 4mM of chromium respectively (Juhnke *et al.* 2002). A rare bacterium called *Ochrobactrum tritici* can resist more than 50mM of chromium. This is because of transposon called TnOtChr which contain chrB, chrA, chrC and chrF genes. Among these chrB and chrA genes shows high resistant capacity than chrF and chrC genes (Branco *et al.* 2008). *Arthrobacter* also reported to have high level of chromate reduction (Diaz *et al.* 2008). In recent studies *Lysinibacillus fusiformis* ZC1 strain was proved to have chrA gene and thus shows heavy metal resistant capacity (He *et al.* 2011). yieF and nitR genes also reported to have chromium resistant capacity. For chromium reduction these genes follow a particular process where the accumulated Cr(VI), present in the cell, induces a chr operon to activate efflux pump containing chrA gene, these genes then rejects the chromium outside the cell and protects it from toxicity. Chromium can reduce by another process where chromate can enter the cell through sulfate uptake mechanism as it is a structural analog of sulfate. If the bacteria contains intracellular chromate reductase enzyme Cr(VI) will automatically reduce to Cr(III) (figure:1).

3. Sulfate Transport Mechanism

As sulfate is a chemical analogue of chromate, it can also be used for Cr-reduction. It enters the bacterial cells through sulfate ABC transporter (Sbp or CysP, the periplasmic sulfate or thiosulfate binding protein, the two inner membrane transport protein, CysT and CysW, the membrane associated ATP binding protein CysA.) which is arranged in operon (Aguilar-Barajas *et al.* 2011). In *S. oneidensis* MR-1, *P. putida* F1, C. metallidurans CH 34 after chromate exposure the sulfate ABC transporter gets up regulated (Brown *et al.* 2006; Thompson *et al.* 2007, 2010; Henne *et al.* 2009b; Monsieurs *et al.* 2011). Studies suggest that *E. coli* K12, *P. putida* F1, *S. oneidensis* MR-1, *C. metallidurans* CH-34 and *Arthrobacter* sp. FB-24 bacteria shows up-regulation in chromate exposure. This is because of the up-regulation of Adenosine 5'- phosphosulfate (APS) at the gene or protein level. This APS activation is catalyzed by ATP- Sulfurylase (Ackerley *et al.* 2006; Brown *et al.* 2006; Henne *et al.* 2009b; Thompson *et al.* 2010; Monsieurs *et al.* 2011). After addition of chromate to the bacterial cultures the components of ABC transporter gets over expressed and the sulfur starvation increases. In this mechanism the Cr-concentration is mainly dependent on the concentration of sulfate. In bacterial culture medium containing cystine or glutathionine blocks the sulfate transport as well as chromate uptake and the sulfer compounds gets activated thus reducing the chromate (Decorosi 2010). On the other hand *C. crescentus* possess a different mechanism for chromium reduction. The sulfate ABC transporter reduces chromate by down regulation. The sulfate reducing bacteria shows chromium resistant capacity hundred times faster than chromium reducing bacteria because of H_2S production. This H_2S first reduces sulfate, then chromate by sulfides (Hu *et al.* 2005).

4. Iron Uptake Mechanism

Some studies also suggest that chromium uptake is also dependent on iron uptake mechanism. A TonB dependent hemoglobin of *P. putida* F1, when exposed to chromium in both LB medium and in minimal medium is up-regulated. But iron uptake is definitely higher in LB medium (Thompson *et al.* 2010). On the other hand *Caulobacter crescentus* also has TonB dependent receptor which possess a slightly different mechanism for chromium uptake. This TonB dependent OM receptor interacts with TonB protein and shows chromium uptake (Hu *et al.* 2005). *S. oneidensis* when given to LB medium, a similar response as *P. putida* F1 was observed against chromium stressed LB medium. A study also reveals that in *S. oneidensis* the iron uptake and Cr(VI) response is depended on activation of a gene called so2426. (Chourey *et al.* 2008). According to studies the Cr(VI) stressed bacterial strains shows a strong up regulation for iron uptake in complex medium after chromate exposure. (Brown *et al.* 2006; Thompson *et al.* 2007).

Table 1: Bacterial genes/proteins involved in resistant mechanism

GENES AND PROTEIN	SPECIES	MECHANISM	REFERENCE
chrA	Pseudomonas aeruginosa	Efflux	Alvarez et al., 1999
chrA	Alcaligenes eutrophus	Efflux	Alvarez et al., 1999
TonB	Caulobacter crescentus	Iron Uptake	Hu et al., 2005
CysA	Shewanella oneidensis	Sulfate Transport	Brown et al., 2006
CysA	Pseudomonas putida	Sulfate Transport	Thompson et al., 2007, 2010
chrB, chrA, chrC and chrF	Ochrobactrum tritici	Efflux	Branco et al., 2008
so2426	Shewanella oneidensis	Iron Uptake	Chourey et al., 2008

CysA	Cupriavidus metallidurans	Sulfate Transport	Henne et al., 2009b ; Monsieurs et al., 2011
TonB	Pseudomonas putida	Iron Uptake	Thompson et al., 2010
chrA	Lysinibacillus fusiformis	Effluix	He et al., 2011

5. Cr (VI) Reduction By Genes And Protein

Cr (VI) can be reduced to Cr (III) by a process called chromate detoxification mechanism, which is not plasmid-associated (Cervantes *et al.* 2001). Two methods are mainly involved-

5.1 Reduction of Cr (VI) under aerobic condition, where NADH or NADPH used as co-factors (Park *et al.* 2000).

5.2 Under anaerobic conditions it can be used as electron acceptor in electron transport chain by some bacteria. These two methods are said to be direct methods (Tebo *et al.* 1998).

Cr(VI) can also be reduced indirectly by Redox intermediate organic compounds like- Amino acid, Nucleotide, Sugar, Vitamins, Organic acid or Glutathione (Myers *et al.* 2000; Robins *et al.* 2013). NfsA / NfsB of *Vibrio harveyi*, NerA of *E. coli* are found to have efficient Cr-reduction capacity. Among them ChrR of *Pseudomonas putida* is found to be best chromate reductase. Some studies also reveal that MtrC and OrcA of *S. oneidensis* MR-1 are terminal reductases of Cr(VI). (figure:1)

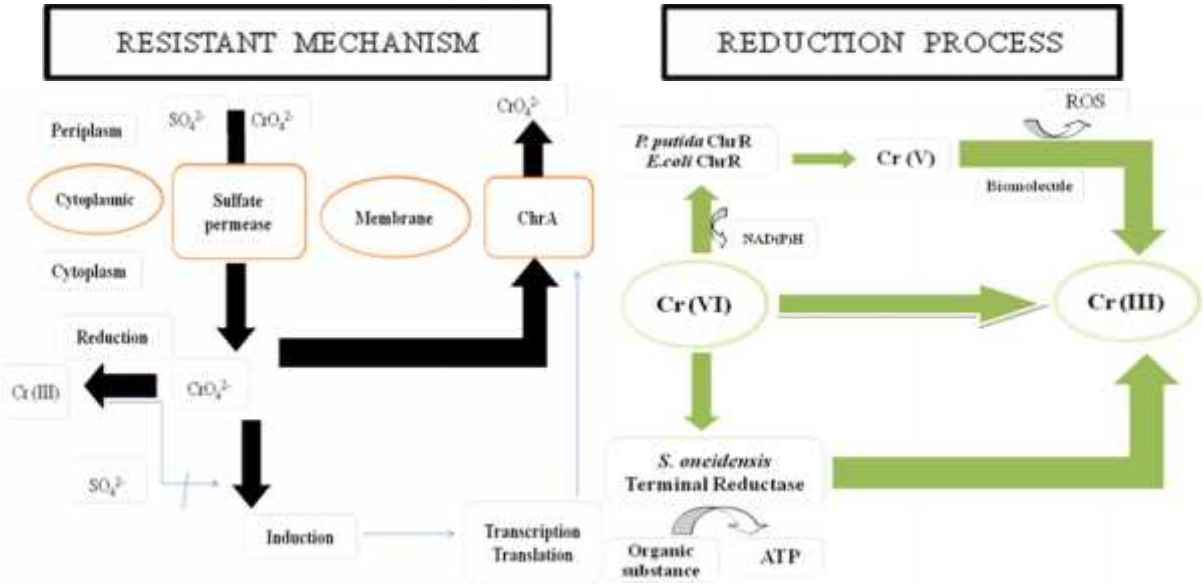

Fig 1: Resistant and reduction mechanism of Cr (VI) by efflux and different bacterial genes.

6. Role of EPS Against Cr(VI)

In bacteria Cr (VI) resistance mechanism also includes the exopolysaccharides (EPS) production within the cells. (Kilic & Donmez 2008; Harish *et al.* 2012). It is biofilm found on the outer surface of cells in most of the bacterial species. The EPS composition can be different in different types of micro-organisms, like *P. aeruginosa* produces alginate as polysaccharide (Jain & Ohman *et al.* 1998) and it was observed that in the presence of chromate the MucD which regulates the transcription of alginate biosynthesis was up regulated. As EPS protects the bacterial cells from the environment it can be said that over production of EPS can be helpful against Cr (VI) stress.

7. Reduction of Chromium By Extracellular Process

In gram (-ve) bacteria this process is followed by two pathways. (Chirwa & Molokwane 2011). One with NADH as electron donor by a soluble reductase and the other one with NADH dehydrogenase under aerobic condition. These enzymes are produced in the cell only when the Cr (VI) is present within the solution (Cheung and Gu 2007). As Cr (VI) reacts with DNA and causes DNA damage and increases the rate of mutation, extracellular reduction process helps to protect the DNA

damage. As a result these bacteria can grow in Cr-contaminated environment. External reduction is always better than internal because it is difficult to remove the resulting Cr(III) from the cells. (Chirwa & Molokwane 2011).

8. Reduction of Chromium By Intracellular Process

Several studies suggest that Cr (VI) reducing enzymes exist within the cells and some components of protoplasm such as NADH, flavoprotein and hemeproteins are also involved in reduction (Ackerley *et al.* 2004). This intracellular proteins mainly reduce Cr (VI) to Cr (V) generates reactive oxygen species (ROS) which causes DNA damage. Bacteria that possess this kind of reduction mechanism are *Bacillus cereus* (Iftikhar *et al.* 2007), *Pannonibacter phragmitetus* LSSE-09 (Xu *et al.* 2012) and *P. putida* (Tripathi & Garg 2013).

9. Membrane Associated Reduction of Chromium

Cr(VI) acts as electron acceptor in the respiratory chains. In *Thermus scotoductus* SA-01 was observed that Cr(VI) was reduced to NADPH by oxidation. Study reveals that a dihydrolipoamide dehydrogenase protein helps in this reduction process. According to chromate reductase assay in alkaliphilic *Bacillus subtilis* a membrane bound enzyme is involved for the reduction process and the decrease of pH with growth of bacteria has a significant role in chromium resistant and reduction mechanism (Mangaiyarkarasi *et al.* 2011). *S. proteamaculans* also shows membrane associated chromate reductase activity (Tahri Joutey *et al.* 2013b).

10. Discussion

As chromium is a non biodegradable toxic heavy metal and discharged regularly from the industries, it is a big threat to the environment as well as human health. Among several oxidation state hexavalent chromium is very much carcinogenic and mutagenic. Many bacterial species have been found which shows the ability to reduce or resist Cr(VI). This mechanism may vary from species to species, which includes efflux mechanism, reduction of Cr(VI) to Cr(III), sulfate transport mechanism, iron uptake mechanism, Cr(VI) reduction by genes and protein, reduction of chromium by intracellular and extracellular process and membrane associated reduction of chromium. Therefore it is necessary to understand the mechanisms involved in chromium reduction and resistant to choose the appropriate one.

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