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In Silico Protein Structure Modeling of Chromate Reductase CR (VI) from Bacillus Thuringiensis

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Abstract

Major factors governing the toxicity of chromium compounds are oxidation state (-2 to 6) and solubility. Cr(VI) compounds, which are powerful oxidizing agents, highly water-soluble and thus tend to be irritating and corrosive, appear to be much more toxic and carcinogenic than Cr(III) compounds. Hexavalent chromium [Cr(VI)] arising as a by-product of numerous industrial processes, is a widespread environmental pollutant. Microbes mainly Bacteria capable of reducing toxic and carcinogenic Cr(VI) to insoluble and less toxic Cr(III), offering promise for an environmentally friendly and approachable solution to chromate pollution. ChrR, a class I chromate-reducing NADH dependent Oxidoreductase from Bacillus thuringiensis is an efficient chromate reducer. In this study, we analyzed the crystal structure of CR(VI) reductase ChrR three dimensional (3D) structure, which is unknown and very essential for advance studies. The stereochemical quality of the best model was validated with 88.7% residues under the favored region from the Ramachandran plot. Altogether, the structure for ChrR has been predicted. In this work, the structural studies pave way for the advance studies of chromate reduction and the methods to be taken and followed for the reduction of chromium involved in the process of healing of chromium pollutants from the environment.

Key words: Hexavalent chromium [Cr (VI)], Homology Modelling, Bacillus thuringiensis

I. Introduction

Chromium is a metallic element that belongs to the transitional series of the periodic table that exists in metallic and alloy states ranging from the oxidation states from -2 to 6 [**13**]. Trivalent chromium (Cr3+) is the stable form of chromium that is a micronutrient present in the environment and in our food, essential for the human body to promote the action of insulin by contributing to the glucose tolerance factor necessary for insulin-regulated metabolism [**14], [02**]. Hexavalent chromium(Cr6+) is another stable form of chromium that is highly water-soluble, toxic and carcinogenic for mammals widely applied in electroplating, stainless steel production, leather tanning, textile manufacturing and wood preservation[**12],[02**]. The tolerance limit for Cr(VI) for discharge into inland surface waters is 0.1 mg·l-1 and in potable water is 0.05 mg·l-1.[**13**]There are various methods for the removal of the toxic form of chromium from the aqueous solution follows physical methods(reduction, ion exchange, electrodialysis, electrochemical precipitation, evaporation, solvent extraction, reverse osmosis, chemical precipitation, and adsorption), chemical methods (reduction by reducing agents like ferrous sulphate and sodium metabisulphite and precipitate out trivalent chromium from solution by precipitating agents[**02],[24**], biological methods include Bioremediation; inventive technology with eco-friendly approach of retrieval and reduction of heavy metals with the help of plants[**19**], Genetically engineered microorganism and genetically modified organism[**25],[09**].

a) Microbial assisted Hexavalent chromium resistance

Microbial assisted technology deals with the microorganisms, which are higher resistance to heavy metals inspite of toxicity of this metal and capable of reducing the toxic level into less toxic. In 1979 the first bacterial was reported with reduction capability and it was Pseudomonas spp. It was outbreak into a new way and followed by a wide range of bacteria including Escherichia coli, Shewanella oneidensis, Deinococcus radiodurans, pseudomonas, and several sulfate reducers have been identified that are capable of carrying out a complete reduction of Cr(VI) to Cr(III).[**09**] Both aerobic and anaerobic microorganisms are capable of reducing heaving metals. Incase of aerobic conditions reduction of Cr(VI) is carried out by soluble enzymes like ChrR and Yief. ChrR catalyzes one electron shuffle followed by 2 electron transfer and reduction of Cr(VI) by the formation of intermediate Cr(III)/Cr(IV) followed by a further reduction to Cr(III). In the absence of oxygen Cr(VI) reduction carried out by membrane-associated enzymes and Cr(VI) act as terminal acceptor of electron transport chain that involves cytochromes [**09**].

Bacillus thuringiensis is an aerobic, gram-positive, endospore-forming soil bacterium in the removal of hexavalent chromium ions because it has a unique ability to produce spore and crystal which is thought to affect its absorption abilities. Hexavalent chromium reduction can either be plasmid-borne or located on the chromosomal DNA. ChR , protein to receive electrons directly from NADH for chromium reduction. The regulation of reduction in an operon structure in Bacillus thuringiensis reduction genes was unregulated by promoter chrI which in turn regulates chrA1 which is a hexavalent resistant gene.ChrA1 and chrI contain putative coding sequences encoding homologs of transposition proteins and that is potentially involved in horizontal transfer events.[**06**]

b) Mechanism of chromium resistance by bacterial cell:

Fig.1 Depicts mechanism of chromium resistance in a bacterial cell: (1) Hexavalent chromium is structurally similar to the sulphate, enters the bacterial cell through sulphate transporter encoded by the chromosomal DNA. (2) Bacterial cells to resist the chromate toxicity, Plasmid DNA encoded the efflux system is used to expel the intracellular chromates outside the cell. (3) Aerobic reduction involves soluble reductase enzyme use NADPH as an electron donor. In anaerobic reduction use electron transport chain pathway by cytochrome b (Cyt b) or Cyt C along the inner membrane, Cr3+ is insoluble and not permeable to enter bacterial cell. (4) Chromate reductase will reduce hexavalent chromium by chromosomal DNA anaerobically in the presence of electron donors. (5) During the redox cyle of hexavalent chromium, Cr5+ produced, it produces oxidative stress by the production of reactive oxygen species (ROS). (6) Protective metabolic enzymes, superoxide dismutase, catalase, and glutathione are secreted to combat the ROS-generated oxidative stress, Some outer membrane proteins are also involved to counter oxidative stress. (7) Cr6+ and Cr3+ will alter the gene expression and also inhibit DNA replication and RNA transcription. (8)DNA repair system gets activated to repair the damaged DNA. [**01**]

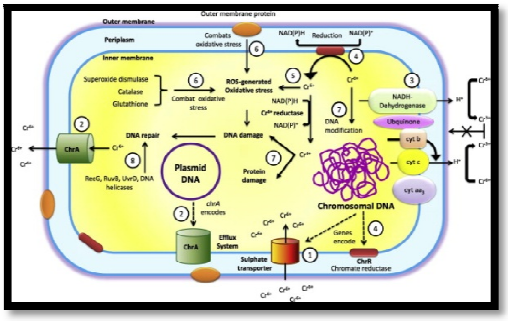

Fig. 1 Overall Mechanism of chromium resistance in bacterial cell

II. Materials and methods

a) Sequence analysis & Modelling:

The suitable template for homology modeling of **Chromate reductase (Cr (VI) reductase)** from Bacillus thuringiensis was investigated by the BLAST (www.ncbi.nlm.nih.gov/blast) search tool and used against Protein Data Bank (PDB) [21]. Modeling of the Chromate reductase (Cr (VI) reductase) ChrR proteins of Bacillus thuringiensis was performed using modeler package swiss modeler [**22**] using the threading approach, considering the low homology of the receptors with that of available structures from the PDB database. To perform the modeling, the templates were searched in the sequence database at NCBI using PSI-BLAST [**02**]. Further, the secondary structures and conserved Domain regions of the target sequences were predicted respectively using GOR and CDD at the NCBI database [**17**]. The homologous sequences thus obtained were aligned with the target sequence and model.

b) Structure Validation:

The 3-D structure of Chromate reductase (Cr (VI) reductase) ChrR of Bacillus thuringiensis was predicted using modeler server by considering the template with high similarity. The best model was chosen based on the stereochemistry quality report generated using PROCHECK [**26**], [**20**], a program that provides an assessment of the stereochemistry of the overall quality of the structure and determines regions to be investigated. The ERRAT tool [**07**] was used for structure verification of non-randomly distributed atoms based on their energetic and geometric effect. The random distribution of atoms are observed in unreliable models and non randomly distributed atoms in a protein structure are expected to be most reliable than unreliable models. VERIFY-3D [**16**], [**23**] was used to check the compatibility of the developed 3D-model of a protein to its own amino acid sequences.

c) Structure estimation:

Selection of an accurate model among alternatives is a crucial step in Homology modelling. SWISS MODEL output page shows model quality in two ways: GMQE (Global Model Quality Estimation) and QMEAN.GMQE is a model quality approximation which is based upon the target template alignment and target-template identification method. GMQE score is between 0 and 1.if the number is close to 1 it indicates higher reliability of predicted model. QMEAN score estimation is based on the geometrical properties of both global and local score. QMEAN score is transformed into Z score which indicates the structure expected from the experimentally determined X-ray structure i.e., “Degree of Nativeness” on the basis of global scale. If the Z score is around 0 it indicates good agreement between model structure and known experimental structure. If the Z score is -4.0 indicated low quality model. [**04**]

III. Results and discussion

Bacillus thuringiensis was able to produce higher degree tolerance to metal, able to produce a higher amount of biomass rapidly, due to this characteristics uptake of metal by bio sorbent material is possible. This study reveals the inexpensive and reliable biosorbing bioagent for bioremediation in the environment [**18**]. The amino acid sequence of Cr(VI) reductase was retrieved in FASTA format from Uniprot Database (http://www.uniprot.org/) with

sequence ID of A0A1B1L3H2 (A0A1B1L3H2_BACTU) having a total sequence length of 244aa.

a) Secondary structure prediction:

The Conserved Domain Database or CD-search service may be used to identify the conserved domains present in a protein sequence and used to help explain about protein function. Figure 2 shows the conserved domain regions of Cr(VI) reductase sequence. Similarly, Secondary Structure of Cr(VI) reductase protein depicted using GOR software in fig. 3.

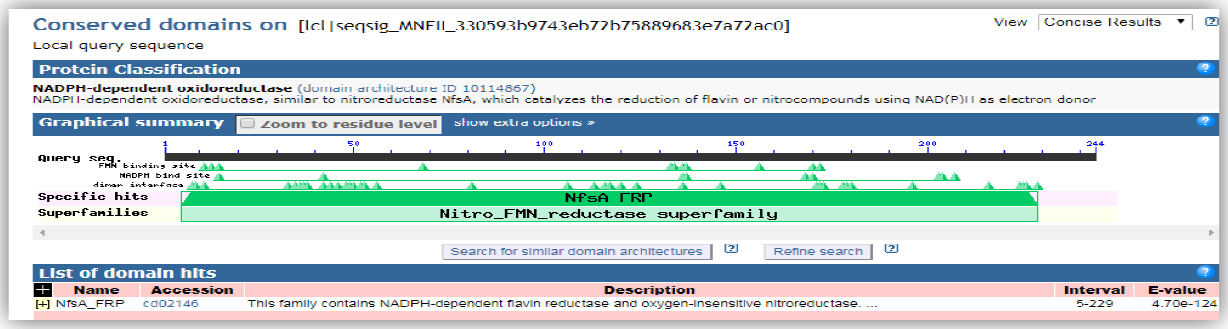

Fig. 2 Conserved domains of Cr (VI) reductase ChR protein, The results are divided into two panels. One is a graphical summary and another one is a table detailing matches. The query sequence of length 244aa is represented in a black bar at the top with a ruler indicating the length above graph summary. Below the query sequence indicating the specific hits to NCBI curated model [**14**].NADPH dependent oxidoreductase domain was observed in the target sequence.

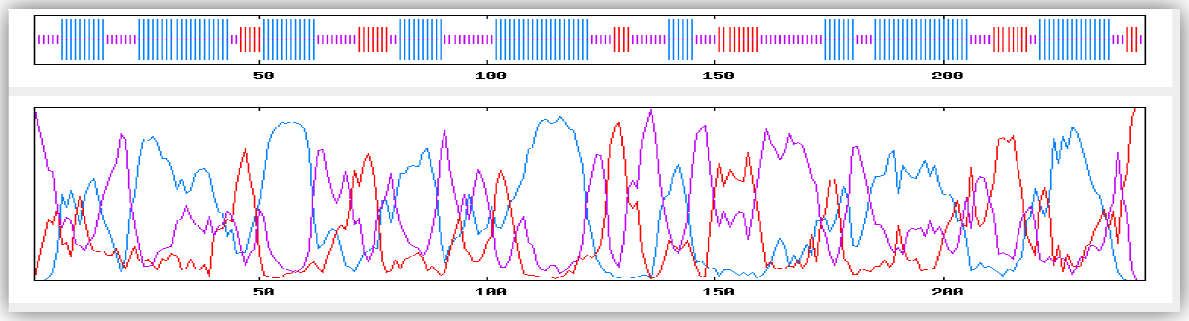

Fig. 3 Secondary structure of Cr (VI) reductase having 244aa length with (a) Alpha Helix (Hh)- 50.41%,(b)Extended strand(Ee) – 14.75%, (c) Random coil(Cc) – 34.84%. The blue line represents alpha helix, Red line indicates extended strand, orange color represents Random coil.

b) Molecular modeling and validation:

Homology or comparative modeling forms a remarkable option for the development of theoretical 3-D protein models, especially when a clear homology is seen between the sequences of the target protein and an elucidated structure. This goes with the assumption that if there is sequence similarity, the tertiary structure of two proteins could be similar. The protein model for Cr(VI) reductase protein was predicted using the SwissPDB Deep viewer server against the chosen template in the Automated mode of the modeling approach, it is 5hdj.1.A namely NfrA1, which had 46.09% identity with the target protein (Fig.4). The quality of the Cr (VI) reductase protein model, as well as the template, was validated through the structural evaluation program PROCHECK (Table 1), and Ramachandran plots provided the analysis of peptide dihedral angles of misfolded proteins into allowed and non-allowed regions.

The analysis revealed that no residues fall under the disallowed region and 88.7% residues fall under the most favored region. The quality of the developed by the model (figure 3) was further validated by the ERRAT score of overall quality factor of A is 92.54 and B is 90.63, indicating the non-randomly distributed atoms, which are considered to be reliable and the resultant higher score greater than 50 is directly proportional to good quality of model. The Verify-3D results of Cr(vi) reductase model of Bacillus thuringiensis showed that 80% of the amino acids had an average 3D-1D score of >=0.2, indicating the dependability of the proposed model. The Z-score value (a measure of model quality as it measures the total energy of the structures) and energy profile of the proposed model were obtained using the same program.

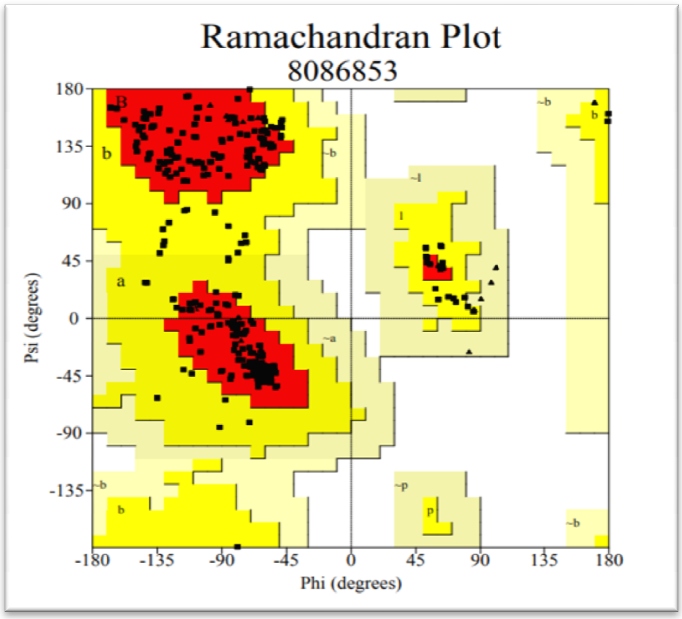

Fig. 4 Ramachandran plot Of Cr(VI) reductase ChrR protein, which validates stereochemical analysis of 3D model quality of protein

Table 1
Results of the structure assessment of predicted model by PROCHECK

Residues in most favoured regions [a,b,l]	88.7%
Residues in additional allowed regions [a,b,l,p]	11.3%
Residues in disallowed regions	0.0%

c) Structure estimation:

```
RasMol> show information
Secondary Structure ... Calcul
Experiment Technique .. THEORI
Number of Chains ..... 2
Number of Groups ..... 485
Number of Atoms ..... 3862
Number of Bonds ..... 3940
Number of H-Bonds ..... 335
Number of Helices ..... 26
Number of Strands ..... 14
Number of Turns ..... 35
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Fig. 6 Rasmol command line of the tertiary structure of Cr(VI) reductase by Homology modeling by swiss PDB software

Fig.5 Quality of estimated model of Cr(VI)reductase Chr protein was estimated using the Q-Mean scoring function. Q Mean score of the model was -0.78 and Z score was 0.79, this shows fine the quality of model because Z score expected to be between 0 and 1.

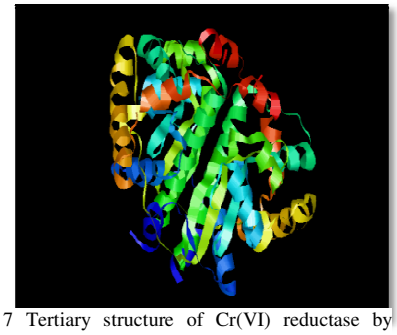

Fig. 7 Tertiary structure of Cr(VI) reductase by Homology modeling by SWISS PDB software visualization in Rasmol

IV. Conclusion:

Bacillus thuringiensis has an important contribution in bioremediation by biosorption of Toxic hexavalent chromium (Cr6+) from the environment by reduction genes upregulated by promoter chrI which in turn regulates chrA1 which is a hexavalent resistant gene. Homology modeling of Cr(VI) Reductase ChrR protein is performed using SWISS PDB DEEP VIEWER, Structure validation is done using RAMPAGE and estimation of the model revealed that it is the highly reliable model according to Z score.

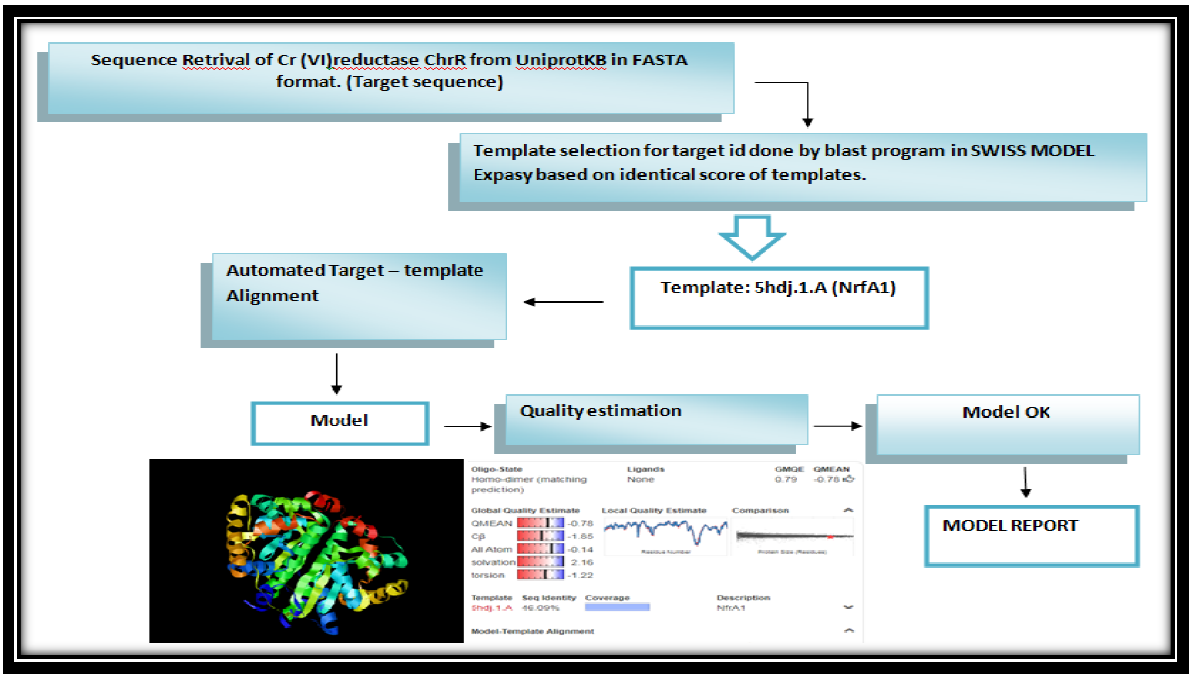

Fig. 8 Overall process of homology modelling of Cr(VI) reductase

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