

***In silico* prediction of metal binding sites in medicinal plants for phytoremediation**

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Abstract:

*Certain plant species and genotypes are able to accumulate large quantities of heavy metal in their shoots. Based on this trait the concept of phytoremediation was developed, i.e. the use of metal hyper accumulating plants for the cleaning of contaminated soil and water. It is a major global problem, which threatens the environment and human life due to toxicity of these metals. Phytoremediation, being more cost-effective and fewer side effects than physical and chemical approaches has gained increasing popularity in both academic and practical circle. The objective of study is to design and analyze metal binding motif against the gene involved in Phytoremediation. Metal binding domains are found in protein that mediate detoxification of metal ion. This is being done on the basis of amino acid residues known to bind metal ion/metal complex in certain medical plants which are able to grow in metal contaminated sites and heavy metal accumulated in these plants do not appear in the essential oil. Recently a lot of interest has been shown by researcher in the *in silico* analysis. Computational analysis include identification of pattern showing high specificity of motif designed for the ions of nickel, lead, molybdenum, manganese, cadmium, zinc, iron, cobalt and xenobiotic compounds by using UniprotKB/ Swissprot and Uniprot KB/ TrEMBL databases. Motifs were also studied against PDB structure. Result of the study suggested that presence of binding site on the surface of protein molecule involved. PDB structure were finally predicted for binding site and further validated through CASTp server. Bioinformatics implications would help in designing strategy for developing transgenic plants with increased metal binding capacity. These metal binding factors help in reducing the possibility of metal movement into food chain.*

Keywords: *Phytoremediation; medicinal and aromatic plants (MAPs); putative metal binding sites.*

Introduction:

The use of plants to degrade, extract, contain or immobilize contaminants from soil and water is Phytoremediation. It is an emerging and cost effective technology^[5]. This technology has been receiving attention lately as an innovative, cost-effective alternative to the more established treatment methods used at hazardous waste sites. Phytoremediation^[14] is the use of living green plants for removal of contaminants from contaminated soil, water, sediments and air. Medicinal and aromatic plants have the ability to accumulate heavy metals and are a good choice for phytoremediation. They have an advantage of not contaminating the food chain with heavy metals as these species are mainly grown for secondary metabolites. Research has shown that heavy metals accumulated by aromatic and medicinal plants do not appear in the essential oil and that some of these species are able to grow in metal contaminated sites without significant yield reduction.

Heavy metal ions such as Cu^{2+} , Zn^{2+} , Mn^{2+} , Fe^{2+} , Ni^{2+} and Co^{2+} are essential micronutrients for plant metabolism^[12]. These ions when present in excess, along with non-essential metals such as Cd^{2+} , Hg^{2+} and Pb^{2+} , can become extremely toxic. Phytoremediation may be applied wherever the soil or static water environment has become polluted or is suffering from ongoing chronic pollution. Various gene families involved in transition/heavy metal transport includes the heavy metal ATPases (HMAs), the natural resistance associated macrophase proteins (Nramps), the cation diffusion facilitator (CDF) family, the ZIPs (ZRT,IRT- like proteins) family, and the Cation anti-porters^[17]. The peptides or proteins helps in detoxification by forming complex with these toxic metals. Plants make two types of peptide metal binding ligands: Metallothioneins (MT's) and Phytochelatins (PC's)^[7]. PCs are enzymatically synthesized peptides, whereas MTs are gene-encoded polypeptides.

The objective of study is to design and analyze metal binding motif against the gene involved in Phytoremediation. This is being done on the basis of amino acid residues known to bind metal ion/metal complex in certain medicinal plants. For this purpose we use a *in silico* analysis high specificity of motif designed for the ions of nickel, lead, molybdenum, manganese, cadmium, zinc, iron, cobalt and xenobiotic compounds by using UniprotKB/ Swissprot and Uniprot KB/ TrEMBL databases^[16]. Multiple sequence alignment is done for finding the conserved regions. These can further classified as the pattern/motifs. Then these motifs are further searched against a database for the particular family. For this purpose pattern/motif database known as PROSITE is used. In the work, specific metal binding motifs have been designed for the ions nickel, lead, molybdenum, copper, manganese, cadmium, zinc, iron, cobalt, aluminum, and magnesium and xenobiotics compounds.

Material and Method:

SWISS PROT: The genes involved in phytoremediation were retrieved from the UniProtKB/Swiss-Prot database belonging to the different sub divisions of Plantae kingdom i.e. Bryophytes, Pteridophytes, Gymnosperms and Angiosperms.

Prosite scan tools: Patterns are searched using this tool .structural residues are obtained from prosite scan tool and then their representative structure is obtained from PDB database.

PDB DATABASE: 3-d structure are obtained from the given database involving the different PDB ID's for different genes

Clustal W: Multiple sequence alignment of representative proteins from different plant species is done which is further helpful in finding the conserved regions.

Metapocket: 2.0 version of this software is used to find the pocket in the desired protein and then active site residues are obtained for the interested pocket. Structure is also visualized by using the Jmol tool.

Pymol: pockets are analyzed and visualized by using pymol visualization tool.

Pockets are located and analyzed by using the following command:

```
#!/usr/bin/python  
from pymol import cmd  
cmd.load("3IPZ.pdb")  
cmd.hide()
```

```
cmd.show("surface")
cmd.color("green")
cmd.load("3IPZ_mpt.pdb","pocket")
cmd.hide("everything","pocket")
cmd.set("sphere_scale","0.5")
cmd.show("sphere","pocket")
cmd.select("MPT","resn MPT and pocket")
cmd.select("PAS","resn PAS and pocket")
cmd.select("QSF","resn QSF and pocket")
cmd.select("PCS","resn PCS and pocket")
cmd.select("FPK","resn FPK and pocket")
cmd.select("SFN","resn SFN and pocket")
cmd.select("GHE","resn GHE and pocket")
cmd.select("CON","resn CON and pocket")
cmd.select("LCS","resn LCS and pocket")
cmd.deselect()
cmd.color("red","resn MPT and pocket")
cmd.color("actinium","resn PAS and pocket")
cmd.color("magenta","resn QSF and pocket")
cmd.color("potassium","resn PCS and pocket")
cmd.color("wheat","resn FPK and pocket")
cmd.color("yellow","resn SFN and pocket")
cmd.color("blue","resn GHE and pocket")
cmd.color("orange","resn CON and pocket")
cmd.color("raspberry","resn LCS and pocket")
cmd.zoom()
cmd.bg_color("white")
```

Results and Discussion

Genes involved in phytoremediation were retrieved through Swiss-prot database. In the present work genes involved in phytoremediation are given in table 1 with their conserved patterns. In the present work, our main goal is to focus on the metal binding motifs involved in the phytoremediation in plants especially in medicinal plants^[16].

Prosite involved signature of protein family involved in metal binding, then multiple sequence alignment is done Using clustal W software. Multiple sequence alignment is helpful in identifying the conserved pattern which is retrieved from prosite server to confirm the pattern of motifs. PDB database is used for obtaining the 3-D structure of genes involved in metal binding with their different PDB id's. Metapocket version 2.0 servers are used to find the active site residues and pockets on the 3-d structure of genes involved in phytoremediation. Metapocket server with default parameters are used which gave the three pockets along with its active sites. Then these active sites are visualized by using Jmol and localization and analyzation of pockets are done using pymol visualization tool with python script extracted from the metapocket results.

Conserved motifs/putative metal ion binding motifs from Prosite database corresponding to heavy metals with respective genes are given in Table 1. The PDB-ids of the representative structures are used in validation are also represented in Table 1. Pymol software shows the cavities as well as residues in proteins where the heavy metals can bind (Fig. 1(a)). Site directed mutagenesis confirm the involvement of residues in certain motifs^[18]. The 3D structure of these motifs were generated and analyzed. The patterns generated out of motifs gives an indication of probable binding sites as visualized through Pymol (Figure 1(b)) for phytoremediation. These were further validated out through Metapocket server. Metapocket web server was used to study surface features, functional regions and

specific roles of key residues of proteins out of the designed motifs. The motifs designed give an insight into the physicochemical properties needed for a protein to perform its function.

Table 1 showing various Genes, metals involved, conserved patterns and PDB-IDs expressed by the genes involved in phytoremediation

Genes	Metals involved	Conserved pattern	PDB ID
OPT		IVDSHfHFD	4IV9
CAX	Ni ²⁺ , Cd ²⁺ , Ca ²⁺	Three conserved patterns are found by using prosite tool LHTAPCGFVVTDAVEpdqPIIYVNTVFEMVTGYRAEEVLGGNCrflqcrgpF AKRRHPLVDSMVVSEIRKCIDEG KILSRLTPRDVASVSSVCRRLYVLTKNEDLWRRV -LIYVNRVFEVFTGYRADEVLGRNCrflqyrdpRAQR	3IPZ
CDF	Pb ²⁺ , Cd ²⁺	The following conserved patterns are retrived from the prosite KDTLEKLVNSEKVVLFMKgtrdfPMCGFSNTVVQILKLNLPFEDVNILEN EMLRQGLKE YSNWPTFPQLYIGGEFFGGCDITLEAFKTGELQEEVEKAMCS- -KILSRLTPRDVASVSSVCRRLYVLTKNEDLWRRV -----LIYVNRVFEVFTGYRADEVLGRNCrflqyrdpRAQR	20EJ 20EK 20EL

Fig. 1(a) Binding cavities in PDB 3IPZ as seen in PYMOL

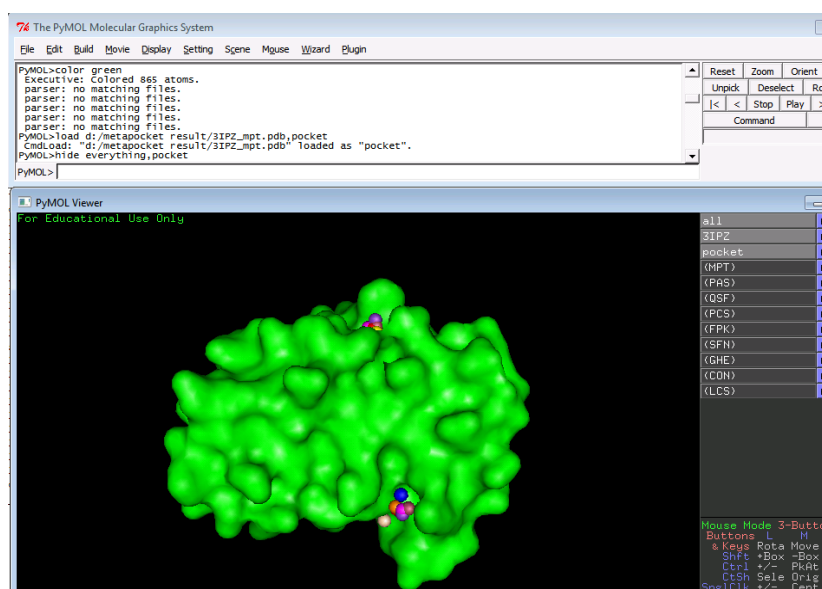
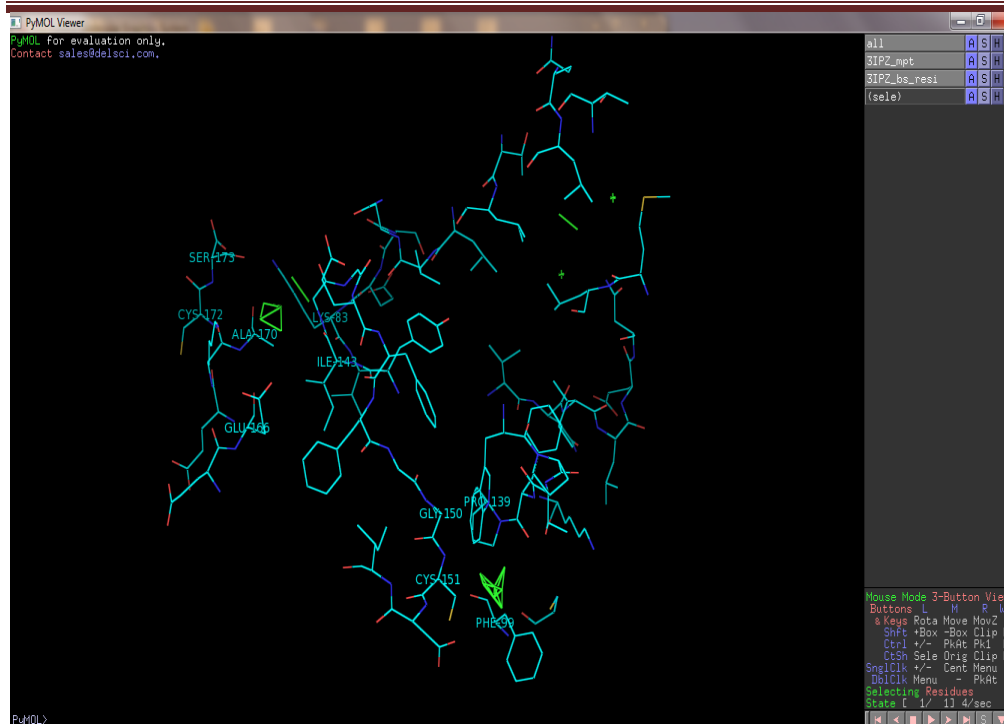


Fig 1(b). PYMOL depicts the binding residues of both the pockets in pdb 3IPZ. PHE99, PRO139, GLY150, CYS151 of pocket-1 and LYS83, ILE143, Glu166, ALA170, CYS172 and SER173 of pocket-2



CONCLUSION:

The motifs demonstrate the signature pattern that are found in the protein structures and are formed by 3-D spatial arrangements of building blocks of proteins i.e. amino acids. All conserved pattern which are used in the present investigation are retrieved from the uniprot having the particular signature sequence for the protein family. The specificity of amino acids plays an important role in the metal binding domains. This knowledge is used developing the metals involved in phytoremediation. The common residues like CYS, PHE of pocket-1 and LYS, ILE, SER and ALA of pocket-2 are present in the motifs also. So they might be the metal binding residues which have the role in binding heavy metals from the soil

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