In silico prediction of Metal Binding Sites in Metallothionein Proteins

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\textbf{ABSTRACT} 
Mining and smelting operations are two important causes of heavy metal contamination in the environment. Contamination of water and soil, soil erosion, and potential risks to human health occurred due to excessive heavy metals dispersion. Heavy metals are a group of elements whose hydro-geochemistry cycles have been accelerated by human activities. Phytoremediation is one of the promising methods for reclamation of soils contaminated with toxic metals by using hyper accumulator plants. Metallothioneins are a group of ubiquitous low molecular mass cysteine-rich intracellular metal binding proteins with a high affinity for divalent heavy metal ions. Metallothionein proteins of three plants \textit{Oryza sativa}, \textit{Triticum aestivum} and \textit{Silene paradoxa} are included in this study. MetalDetector web server used in this study to predict metal binding sites of these three proteins. Our results show that \textit{Oryza sativa} and \textit{Silene paradoxa} are more suitable plants than \textit{Triticum aestivum} for metal chelating.

\textbf{Introduction} 
Metals occur naturally in soils in small amounts. Human activities like metal industry and transportation, also can release metals into the environment. Metals like zinc, iron and copper are essential micronutrients required for a wide range of physiological processes in all plant organs for the activities of various metal-dependent enzymes and proteins. However, they can also be toxic at elevated levels. Metals like arsenic, mercury, cadmium and lead are nonessential and potentially highly toxic.\textsuperscript{(Krämer et al., 2005)} As a consequence of metal deposition, soil organisms may fail to survive in contaminated environments, or their function in decomposition processes may be reduced. Soft bodied animals like earthworms are exposed to metals directly through their skins as well as via their diet (Eijsackers 1998). Several studies have reported reduced species diversity, density and biomass in lumbricids due to increased metal concentrations in soil (Bengtsson and Tranvik., 1989, Spurgeon and Hopkin., 1999, Lukkari et al., 2004). However, some earthworm species, including D. octaedra, can be found in metal contaminated soils (Bengtsson et al., 1983, Lukkari et al., 2004). The contamination of the environment by toxic metals is a threat for “Man and biosphere”, and reduce agricultural productivity and damage the health of the ecosystem (Andrews GK., 2000). This problem is being solved by using different methods such as “green technology” involving metal tolerant plants (BoucheN and BouchezD., 2001). The use of tolerant plants for remediation of soils could be a low cost and useful method. Phytochelatins (PCs) and Metallothionins (MTs) are the best characterized plants proteins that mediated metal chelating (Clemens S et al., 1999). Remediation of metal-contaminated soils is notoriously hard. The cost of soil remediation is variable and depends on type of contaminance, site conditions and the extent of contamination. Cleaning of metal-contaminated soils via conventional engineering methods can be prohibitively expensive (Salt et al., 1995). The use of plants for cleaning of contaminated environments is not new. 300 years ago plants were used in treatment of wastewater (Hartman, 1975). \textit{Thlaspi caerulescens} and \textit{Viola calaminaria} are the first plant species documented for accumulation of metals in leaves. (Baumann, 1885). Phytoremediation is one of the promising methods for treatment of contaminated soils, water, or air by using hyper accumulator plants that are able to degrade or aggregate the metal pollutants without the need to excavate the contaminant material and dispose of it elsewhere. (Baker et al., 2000; Ghosh and Singh., 2005; La’zaro et al., 2006). Today research for identifying metal resistant plants is expanding. Baker and Brooks in 1989 identified more than 400 plant species belonging to 45 plant families from temperate to tropical regions with the ability to tolerate and hyper accumulate trace elements. Hyper
accumulator plants can play a critical role in remediation of contaminated soil and water. MTs and PCs are two types of peptide metal binding ligands make by plants. MTs for the first time were identified as Cd-binding proteins in mammalian tissues. (Kagi et al., 1991). MTs are a class of cysteine-rich, heavy metal binding molecules encoded by a family of genes (Salt DE et al., 1996). Phytochelatins are small, cysteine-rich peptides capable of binding heavy metal ions via thiolate coordination. Phytochelatins are assumed to be involved in the accumulation, detoxification, and metabolism of metal ions such as cadmium, zinc, copper, lead, and mercury in plant cells (Grill et al., 1985). The use of plants for remediation of metal contaminated soils is a great promising approach for replacing the old methods. Phytoremediation is the use of plants to make soil contaminants nontoxic (Chaney et al., 1997). Plants have developed a complex network of highly effective homeostatic mechanisms that serve to control the uptake, accumulation, trafficking, and detoxification of metals. Components of this network have been identified continuously, including metal transporters in charge of metal uptake and vacuolar transport; chelators involved in metal detoxification via buffering the cytosolic metal concentrations; and chaperones helping delivery and trafficking of metal ions. In this research we try to find the better Metallotionin and so we introduce the better plants for using for phytoremediation. MetalDetector server (Lippi et al., 2008) that we use in this research can also use for finding proteins with high tendency to metals for cloning their genes in plants and bacteria for remediating the soils with this new transformed species.

Materials and Methods

Three complete sequences of Oryza sativa, Triticum aestivum and Silene paradoxa are included in this study. Sequences of Metallothionein from each plants were obtained from NCBI - GenBank, Protein Data Bank (PDB). The accession number of these three protein sequences are AAD02475.1 for Oryza sativa, AAO12853.1 for Silene paradoxa and AAP80616.1 for Triticum aestivum. Multiple sequence alignment programs - ClustalW, T-Coffee and Multalin programs were used to confirm and validate the reliability of the data. For identifying the metal binding site of these proteins we used MetalDetector server (http://metaldetector.dsi.unifi.it). The web server MetalDetector (Lippi, M et al, 2008) classifies histidine residues in proteins into one of two states (free or metal bound) and cysteines into one of three states (free, metal bound or disulfide bridged). Cross-validated performance assessment indicates that this server predicts disulfide bonding state at 88.6% precision and 85.1% recall, while it identifies cysteines and histidines in transition metal-binding sites 79.9% precision and 76.8% recall.(Andrea Passerini et al., 2011). Giving protein sequences in FASTA format to MetalDetector, the Histidin and Cystein residues that bind to Metals were predicted and the results analysed. The web server MetalDetector classifies histidine residues in proteins into one of two states (free or metal bound) and cysteines into one of three states (free, metal bound or disulfide bridged) The main purpose of MetalDetector is to make the predictor available online as a web application.(Marco et al., 2008)

Results and Discussion

Multiple sequence alignments by ClustalW indicate there are two major sections for the aligned protein residues (Figure. 1). The results of MetalDector (Figure. 2) Show that Metallothionein of Oryza sativa binds metals in three sites and the Metallothionein of Silene paradoxa binds metals in four sites while Metallothionein of Triticum aestivum does not bind metals.

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CCGSACKCGSGCCHKMFDPASAS-SGSASLILGVAPK-------------GCKCGDNCQCNPLTCK
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CCGSACKCGSGCCHKMYEMDEGVNTSSQTLIMGVAFS-------------GCKCGENCSNCNPTCK
---
CCGSACKCGSCNCCKKYPDLEEEKSSSSTKATVVLGVAFE-------------CCGSCGCRCSNCNTCK
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Fig. 1. Multiple sequence alignment of protein Metallothionein from three plants Oryza sativa, Triticum aestivum and Silene paradoxa performed by ClustalW
Phytoremediation can be defined as the efficient use of plants to remove, detoxify or immobilize environmental contaminants in a growth matrix (soil, water or sediments) through the natural biological, chemical or physical activities and processes of the plants. The plants can be subsequently harvested, processed and disposed. Heavy metal ions such as Cu\textsuperscript{2+}, Zn\textsuperscript{2+}, Mn\textsuperscript{2+}, Fe\textsuperscript{2+}, Ni\textsuperscript{2+} and Co\textsuperscript{2+} are essential micronutrients for plant metabolism. These ions when present in excess, along with non-essential metals such as Cd\textsuperscript{2+}, Hg\textsuperscript{2+} and Pb\textsuperscript{2+}, can become extremely toxic (Ashok et al., 2009). Phytoremediation is an emerging technology based on the use of plants to clean up polluted sites. Phytoextraction is the removal of metals or other pollutants from contaminated soils whereby the metal is extracted from the soil, and then translocated to and concentrated in the harvestable parts of the plant. Phytoremediation technologies are becoming recognized as cost-effective methods for remediating sites contaminated with toxic metals at a fraction of the cost of conventional technologies, such as soil replacement, solidification and washing strategies. Apart from accumulating high levels of metal and translocating it to the harvestable segments of the plant, a plant suitable for phytoextraction should grow rapidly and reach a high biomass. Decontaminating sites in a reasonable number of harvests requires plants that produce both a high biomass and accumulate at least 1–3% metal, by dry weight. The results show that as the Metallothionein proteins of *Oryza sativa* and *Silene paradoxa* have more binding site for metals than *Triticum aestivum*, Metallothionein protein so *Oryza sativa* and *Silene paradoxa* are good plants for phytoremediation of soil contaminated with heavy metals.

**Conclusion**

Today, bioremediation is primarily explored for purifying metal contaminated waste. The ultimate goal is to use microorganisms or plants in the biosorption of metal-polluted waste, waste streams and soils. Metalloproteins are a diverse class of proteins that bind one or more metal ions in their native conformation. Metal atoms play a wide range of structural, regulatory or catalytic roles which are critical to protein function (Degtyarenko et al., 2000) In order to find the best plants for using for metal bioremediation we compare the Metallothionein of three different plants. MTs have the capacity to bind both physiological (such as zinc, copper, selenium) and xenobiotic (such as cadmium, mercury, silver, arsenic) heavy metals through the thiol group of its cysteine residues, which represents nearly the 30% of its amino acidic residues.

**References**


