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GENETIC ENGINEERING TO EXPRESS METAL BINDING PROTEINS AND PEPTIDES: IMPLICATIONS FOR BIOREMEDIATION

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ABSTRACT

Plants and microbes respond to heavy metal toxicity in different ways. Such responses include immobilization, exclusion, chelation and compartmentalization of the metal ions and the expression of more general stress response mechanisms such as ethylene and stress proteins. Understanding the molecular and genetic basis for these mechanisms will be an important aspect of developing plants as agents for the phytoremediation and microbes for bioremediation of contaminated sites. One recurrent general mechanism for heavy metal detoxification in plants and other organisms is the chelation of the subsequent compartmentalization of the ligand-metal complex. A number of metal-binding ligands have now been recognized in plants Peptide ligands include the metallothioneins (MTs), small gene-encoded, Cys-rich polypeptides. Our current review justified the functions and expression of MTs and PCs in plants and microbes. In contrast, the phytochelatins (PCs), the subject of this Update, are enzymatically synthesized by Cys-rich peptides. The expression of metal-binding proteins or peptides in microorganisms and plants, in order to enhance heavy metal accumulation and/or tolerance has great potential. Several different peptides and proteins have been explored. We have picked-up cadmium (Cd), because of the significant importance of this metal and its global presence in many food materials.

Key words : Phytoremediation,Bioremediation,Detoxification,Metallothioneins,Phytochelatins.

INTRODUCTION

Contamination of soil, aqueous streams and ground water with toxic metals poses a major environmental problem and a serious danger to human health that still need an effective and affordable technological solution (Odawara F.et al, 1995. Heavy metals are difficult to remove from the environment and unlike many other pollutants cannot be chemically or biologically degraded and are ultimately indestructible. Today, many heavy metals constitute a global environmental hazard. Various physical, chemical and biological processes are already being used to remediate contaminated soil.

Bioremediation strategies have been proposed as an attractive alternative technology to their low cost and high efficiency. Thus the use of microorganisms and plants for the decontamination of heavy metals has attracted growing attention because of several problems associated with pollutant removal using conventional methods. Lower cost and higher efficiency at low metal concentrations make biotechnological processes very attractive in comparison to physicochemical methods for heavy metal removal. For example, environmental pollution by Cd, arising mainly from mining and smelting, dispersal of sewage sludge and the use of phosphate fertilizers, is

increasing (Raskin.I. et al, 1994). Phytoremediation is an emerging technology based on the use of plants to clean up polluted site. Phytoextraction is the removal of metals or other pollutants from contaminated soils where by the metal is extracted from the soil and then translocated to a concentrated in the harvestable parts of the plant. Agricultural soils are mainly contaminated with Cd from the excessive use of phosphate fertilizers, dispersal of sewage sludge and atmospheric deposition. Cd is readily taken up by many crops including cereals, potatoes, vegetables (leafy and root) and fruits. Bacteria and higher organisms have developed resistance mechanisms to toxic metals to make them innocuous (Stefanov I.et al,1997). Organisms respond to heavy metal stress using different defence systems, such as exclusion, compartmentalization, making complexes and the synthesis of binding proteins such as metallothioneins (MTs) or phytochelatins (PCs). The over expression of metal binding proteins have been widely exploited to increase the metal binding capacity, tolerance or accumulation of bacteria and plants. Modification in the biosynthesis of PCs in plants has recently been accomplished to enhance the metal accumulation whereas in bacteria, various peptides consisting of metal-binding amino acids (mainly histidine and cysteine residues) have been studied for enhanced heavy metal accumulation by bacteria.

Mechanisms of heavy metal (cadmium) tolerance in plants:

Cadmium (Cd) which is strong phototoxic heavy metal, resist plant growth, and can leads to plant death. Stunting and chlorosis are the main symptoms of $Cd^{(2+)}$ toxicity to the plants .The $Cd^{(2+)}$ tolerance reflect in Plant by developed some functions such as, chelation with phytochelatins (PCs), cell wall binding, enrichment in leaf trichomes and compartmentation of $Cd^{(2+)}$ in the vacuole (Mejare M. el al,1998). $Cd^{(2+)}$ tolerance in plants are closely related with the processes of antioxidative response, sulphur metabolism, $Cd^{(2+)}$ transport across plasma and the vacuole membrane of plant. $Cd^{(2+)}$ stress responded by the processes like sulphur uptake, assimilation and sequential sulphur metabolism in plant (Mauro JM. et al,2000) . Under $Cd^{(2+)}$ stress the expression of sulphur transporters with diverse similarity was changed in dissimilar ways, and high expression of ATP sulfurylase (APS) and adenosine 5' phosphosulfate reductase (APR), which may help to keep the supply of $S^{(2-)}$ for cysteine (Cys) synthesis. The efficiency of Cys synthesis may role in $Cd^{(2+)}$ detoxification, and in some $Cd^{(2+)}$ treated plants the up-regulated expression of Ser acetyltransferase (SAT) and O-acetyl-ser (thiol)-lyase (OASTL) has been found(Sousa C.et al , 1996b) . Reduced glutathione (GSH) is an vital antioxidant as well as precursor of PCs, glutamylcysteine synthetase (GCS) and glutathione synthetase (GS) catalyze GSH synthesis from Cys, overexpression of the two enzymes can be use for improvement of $Cd^{(2+)}$ tolerance in plants.(Valls et al,1998).PCs are more important $Cd^{(2+)}$ chelators than metallothioneins (MTs) in plants, and expression of phytochelatin synthase (PCS) responds to $Cd^{(2+)}$ stress. For $Cd^{(2+)}$ tolerance the Plant antioxidative system also contributes a significant role. In recent years Some genes encoded metal transporters with $Cd^{(2+)}$ substrate specificity at plasma and vacuole membranes,have been isolated and characterized. These genes contribute vital roles in $Cd^{(2+)}$ translocation, allocation, and compartmentation in plants(Hou Y.et al,1988) .Although the great progresses made in the field in recent years, there are still some issues which need further exploration, for example the rhizosphere activation and root adsorption to soil $Cd^{(2+)}$, $Cd^{(2+)}$ translocation to fruit and seed, the detail of signal transduction and the responses of gene regulation to $Cd^{(2+),}$ $Cd^{(2+)}$ trafficking in xylem and phloem, and the possible presence of a high-affinity $Cd^{(2+)}$ transporter in Cd hyperaccumulators.

Potential mechanisms that govern heavy metal tolerance in plant cells are: (1) metal binding to the cell wall; (2) reduced transport across the cell membrane; (3) active efflux; (4) compartmentalization and (5) chelation. The widespread mechanism is chelation based on induction of metal-binding peptides and the formation of metal complexes. For the formation of complexes with heavy metals in plants the

members of the third class of MTs, the PCs, are accountable (Grill E. et al, 1997). These metal-binding peptides are enzymatically derived and synthesized on exposure of the cell to toxic metals. PCs are induced in some fungi as well as all autotrophic plants analysed so far. The structure of PCs is (γ -Glu-Cys)nX, in which X is Gly, γ-Ala, Ser or Glu and n = 2–11 depending on the organism, even though the most common forms have 2–4 peptides. The biosynthesis of PCs is induced by many metals including Cd, Ag, Hg, Ni, Cu, Pb, Au and Zn; but, Cd is by far the strongest inducer.The metal bind to the constitutively expressed enzyme, γ-glutamylcysteinyl dipeptidyl transpeptidase (PC synthase), and advantages to activating it to catalyse the change of glutathione (GSH) to phytochelatin. Glutathione which is the substrate for PC synthase, is synthesized from its component amino acids in two steps. The first step is catalysed by γ -glutamyl- Cys synthetase (γ -ECS) and the second one by glutathione synthetase (GS). γ -ECS is feedback regulated by glutathione and is depend on the availability of cysteine. MT-like genes have been isolated from a number of plant species including maize, Brassica napus, tobacco, soybean, wheat, and rice but their role in metal detoxification has yet to be recognized (Murooka,Y.et al,1987) . Type I MT-like genes are found mainly in the roots whereas type II MT-like genes are expressed predominantly in the leaves.

Expression of heterologous MTs in bacteria:

For enhancing the metal tolerance, sequestration or accumulation of bacteria, the high metal-binding capacity of MTs has been broadly exploited. The MTs which receive establish from various wellsprings acquire been expressed intracellularly in Escherichia coli including monkey MT, human MT-II(Sayers Z. et al,1993), mouse MT-I, yeast MT (Barka T.et al,1988). In many cases, the problem like stability and short life of the expressed heterologous proteins were encountered. The problem was associated to the foremost cysteine meaning of MTs which force interfere with cellular redox pathways in the cytosol.The small molecule of MT can be stabilized by fusion to large molecules, like β-galactosidase .The human MT which fused to β-galactosidase enhanced uptake of Cd by the recombinant *E.Coli.* The expression of a fusion protein of human MT and araB increased the stability of MT *Escherichia coli* expressing the fusion protein accumulated five times more Cd than control cells. In this process no increased metal resistance was observed but in the case of human MT-II but when expressed intracellularly in *E. coli,* an increase in the metal resistance of the cells found (Wagner GJ. 1993). The metal-binding proteins have been anchored to the LamB further the OmpA, proteins that period the exterior membrane, or to PAL,a protein that is anchored to the peptidoglycan crust. MTs ,Yeast (CUP1) and mammalian (HMT1A) when expressed on the surface of *E. coli* as fusions to LamB improved the metal binding capacity of the cells from 15 to 20-times.(Jacobs FA. et al,1989) 19 .in the case of *Neurospora crassa* MT was expressed in 1 to 12 repeats as a fusion with the maltose-binding protein in the periplasm of *E. coli* and in this process the Cd-binding capacity was increased ten times with one repeat and a maximum 65-times with nine number of repeats.

When only the α- or β-domains of a mammalian MT were expressed on the cell surface as fusions to LamB ,the metal accumulation of *E. coli* cells was compared (Kotrba P.et al, 1999) . Surface expression of the α-domain alone has shown to be more useful in metal accumulation in the cell as compared to an expression of the entire MT. The cells that express α-domain exhibited a 17-time increase in Cd accumulation by cells compared with control cells,whereas cells that express the β-domain exhibited a three-fold increase in Cd accumulation. Additionally, when grown in 30 μM cadmium chloride ($CdCl_2$), cells expressing in α-domain accumulated more Cd than cells expressing the entire MT protein. The benefit of diverse exterior membrane proteins (OMPs) as supports for MTs at the cell surface has been investigated by Valls et al., who originate LamB to be the elite candidate for sequestering Cd (Valls A.et al, 1998). In addition, if non-viable cells are to be used for bioremediation and if the biomass is to be recycled by desorption, surface display is preferred.

Table 1. Metal binding peptides and proteins expressed on the surface of *Escherichia coli* and their effect on the cells upon Cd exposure.

Peptide/protein	Expression site	Effect	Reference
CdBP(HSQKVF)	OM,Ompa	Increased Cd tolerance capacity	Clemens S., et al.
CP(GCGCPCGCG)	OM,LamB	Four fold increase in Cd accumulation	Kotrba P.,et al.
His6, single or tandem expressed	OM, LamB	Five-and11-fold increase in Cd accumulation	Sousa C., et al.
HP (GHHPHG)2	OM, LamB	Three-fold increase in Cd accumulation	Dorhalc F.D.,et al.
Human MT	OM, Lpp	66-fold increase in Cd accumulation	Sayers Z.,et al.
Mammalian MT	OM, LamB	15–20-fold increase in Cd accumulation	Sousa C., et al.
MT α-domain	OM, LamB	17-fold increase in Cd accumulation	Sayers Z.,et al.
(CGCCG)3	OM, LamB	Ten-fold increase in Cd accumulation	Pazirandeh M.,et al.

Abbreviations: OM, outer membrane; LamB, cysteine-containing peptide; HP, histidine-containing peptide, Calcium-binding; Lpp, protein; OmpA.

Table-2. Expression of metallothioneins in transgenic plants.

MT source	Growth conditions	Cd tolerance/accumulation	Reference
Human MT-II	MS-media	Growth of root and shoot unaffected up to 100 μM.	Nedkovska M.et al.
Twelve repeated α-domains of human liver MT-IA	MS-media	Growth of root and shoot unaffected up to 100 μM.	Fordham-Skelton A.P.,et al.
Human-MT-β-glucuronidase fusion	In vitro grown seedlings	60–70% lower Cd accumulation in shoots of transgenes than in control	Elmayan T.,et al.
Human MT-II	Field grown	73% less Cd in leaf lamina than in controls. Overall Cd accumulation not affected compared with controls.	Dameron C.T., et al.

Regulation and expression of metal-binding peptides:

A number of metal-binding peptides have been studied for increasing the Cd resistance or accumulation by *E. coli* cells. MTs and PCs, which are naturally occurring Cd-binding proteins and peptides, are very rich in cysteine residues. Additionally, histidines are known to have high affinity for transition metal ions such as $Zn^{2+,}$ $Co^{2+,}$ Ni^{2+} and Cu^{2+}, MZn^{2+} bears a similarity to Cd^{2+} due of its position in the periodic table (Wagner GJ.et al, 1993). So, various peptides having different sequences of cysteines or histidines have been exploited for

binding Cd. In Recent year, metal-binding peptides,containing either histidines $(GHHPHG)_2$ (HP) or cysteines (GCGCPCGCG) (CP) were engineered to LamB and expressed on the surface of *E. coli* (Kemper MA. et al,1993) .

Surface display of CP increased the bioaccumulation four-times and HP two-times. Expression of a repetitive cysteine rich metal-binding protein $(CGCCG)_3$ as a fusion with the maltose-binding protein in *E. coli* improved Cd^{2+} and Hg^{2+} binding 10-times. In addition, a His^6 peptide has been expressed on the surface of *E. coli* as a fusion to the OMP LamB. This resulted in a five-time increase in Cd accumulation when the peptide was expressed with single copy and an 11-time increase when expressed in tandem. But, no increase in metal tolerance was found. The fusion of hexa peptide in quadruplicate to the N-terminal of either lactate dehydrogenase (LDH) or green fluorescent protein (GFP) when expressed intracellularly resulted in increased tolerance of the cells to toxic Cd concentrations. The expression of hexa-peptide containing four Histidines(SYHHHH) in *E. coli* did not render these cells more tolerant to Cd than those expressing CdBP on the cell surface. This is sign that a peptide consisting of only one histidine in combination with other specific amino acids might be just as fine as a multi-histidine peptide for a metal chelator. A summation of Cd accumulation and tolerance obtained when expressing metal binding peptides and MTs on the surface of *E. coli* is given in Table 1. In few cases the expression of Cd-binding peptides, or proteins in *E. coli*, has shown a more tolerance to Cd, and an better accumulation of the cells. However, it is possible that, for the intracellular expression of metal-chelating peptides, the use of peptides devoid of cysteines is less toxic to the cell compared with expression of cysteine-rich MTs. This may be due to, that the peptide is under the control of a constitutive promoter and not regulated by the metal ion concentration within the cell. Metal-binding peptides and MTs have primarily been exploited to enhance the Cd tolerance or the accumulation of *E. coli* cells. It may be of great significance to evaluate Cd-binding peptides and proteins, engineered into more environmentally strong bacteria, like *Pseudomonas*, for their prospective use in the area of bioremediation.PCs are the main detoxifiers by chelation of the metal. For enhancing heavy metal tolerance and accumulation in plants,modification of the enzymes that are involved in the synthesis of glutathione and PCs, might be a excellent approach.

Genetic Engineering of plants:

In genetic engineering of plants, a foreign piece of DNA is stably inserted into the genome of a cell, which is regenerated into a mature transgenic plant. The piece of DNA can emanate from any organism, from bacteria to mammals. The foreign DNA customarily contains two genes, one a resistance gene utilized for cull after transformation, another, gene of interest. Each gene is coupled to a plant promoter, ascertaining the formation of the gene product (conventionally a protein) in the plant. When the transformed plant is propagated, the foreign gene is inherited by its progeny. The foreign part of DNA may be transferred to the plant either via a particle gun, for which the DNA is coated on to metal particles and shot into the plant tissue. or, a soil bacterium like *Agrobacterium* that makes a living by inserting the part of foreign DNA (called T-DNA) into a plant cell and feeding off of the gene products engendered by the plant. The *Agrobacterium* T-DNA genes can be superseded by genes of interest, which are then inserted into the plant by *Agrobacterium* infection. Most plants need to be grown as undifferentiated callus tissue culture in order to be transformed. After the transformation, mature plants are regenerated from the tissue culture using shoot inducing plant hormones. The gene product can be targeted to certain cellular compartments (e.g. chloroplast, vacuole, mitochondrion, or apoplast) by integrating concrete targeting information in the gene construct. Often, constitutive promoters such as the 35S-cauliflower mosaic virus promoter are used, that direct expression in all tissues and at all times. However, the expression pattern of the gene may withdrawl be programmed to be only in certain tissue types (e.g. roots, vascular tissue, shoot), or under certain environmental conditions (stress-induced, light-induced), by

means of different promoters (Kramer U et al,1996) . Besides overexpressing a gene, it is withal possible to repress the expression of an endogenous gene, by inserting a replica of that gene in inversion orientation (antisense technology).

The role of heterologous MTs in transgenic plants:

Transgenic plants express MTs enhancing Cd tolerance and Cd accumulation (Table 1). The stable integration of metallothioneins in transgenic plants appeared in 1989. Overproduction of various metal chelator protein affected on plant metal tolerance and accumulation. Several researchers have over expressed the metal-chelating proteins metallothioneins (MTs). The expression of the human MT2 gene in tobacco, or oil seed rape resulted in higher Cd tolerance in the seedling level. Similarly, the expression of the mouse MT1 gene in tobacco led to enhanced Cd tolerance at the seedling level. The most pronounced effect of MT over expression was observed by Grill E *et al.* (1989), who over-expressed the yeast gene *CUP1* in cauliflower, leading to a 16-fold Cd tolerance and accumulation. Thus, this overexpression of MTs is a promising approach to enhance Cd/Cu tolerance and accumulation.

PCs have the general structure (g-Glu-Cys)n-Gly:

Early analyses demonstrated PCs consisted of only the three amino acids: Glu, Cys and Gly with the Glu, and Cys residues linked through a g-carboxylamide bond. PCs are the family of structures with incrementing repetitions of the g-Glu-Cys dipeptide followed by a terminal Gly; (g-Glu-Cys)n-Gly, where $n \geq 11$, but is generally in the range of 2 to 5. PCs have been identified in a wide variety of plant species and in some microorganisms (Elmayan T. et al 1994).They are structurally cognate to glutathione (GSH; g-Glu-Cys-Gly) and were surmised to be the products of a biosynthetic pathway. In addition, a number of structural variants, for example, (g-Glu-Cys)n-b- Ala, (g-Glu-Cys)n-Ser, and (g-Glu-Cys)n-Glu, have been identified in some plant species (Silver S,1992).

PCs are synthesized from GSH:

Numerous physiological, biochemical, and genetic studies have attesed that GSH (or, in some cases, cognate compounds) is the substrate for PC biosynthesis. Such studies have utilized a variety of plant species, sometimes as intact plants, but often in case of in vitro cell cultures. Early cell culture studies described that induction of PCs in the presence of Cd coincided with a transient decrease in levels of GSH. Furthermore, the exposure of either cell cultures or intact plants to an inhibitor of GSH biosynthesis, but thionine-sulfoximine, conferred incremented sensitivity to Cd with a corresponding inhibition of PC biosynthesis. This could be inverted by the integration of GSH to the growth medium. By far the most detailed characterization of the pathway of PC biosynthesis has emanate from studies in the fission yeast (*Schizosaccharomyces pombe),* and in *Arabidopsis*. Genetic studies have attested GSH deficient mutants of the fission yeast and *Arabidopsis* are additionally PC deficient and hypersensitive to Cd. In particular, the *cad2-1* mutant of *Arabidopsis* is partially deficient in GSH and in g-glutamyl-Cys synthetase (GCS) activity, the first of the two GSH biosynthetic enzymes. The *cad2-1* mutation is a 6-bp deletion within an exon of the GCS gene affecting residues in the vicinity of the postulated active site of the enzyme (Mehera R.K. et al.,1998).

PC Synthase is activated by heavy metal ion:

An enzyme activity from cultured cells of *Silene cucubalis* that synthesized PCs from GSH by transferring a g-Glu-Cys moiety from a donor to an acceptor molecule,was first identified by Grill et al(1989) . The reaction involved the transpeptidation of the g-Glu-Cys moiety of The enzyme is an 95,000-*M*r tetramer with a *K*m of 6.7 mm for GSH. In vitro, the activity of the partially purified enzyme was active only in the presence of metal ions. The best activator tested was Cd followed by Ag, Bi, Pb, Zn, Cu, Hg, and Au cations. GSH on to initially a second GSH molecule to compose PC2 or, in later stages of the incubation, on to a PC molecule to engender an n 1 1 oligomer. This g-Glu-Cys dipeptididyl transpeptidase has been designated PC synthase. These metals also induce PC biosynthesis in

vivo in plant cell cultures. In in vitro reactions, PC biosynthesis continued until the activating metal ions were chelated either by the PCs formed or by the addition of a metal chelator such as EDTA (.Lovley DR and Coates JD, 1997).This mechanism is the autoregulator PCs biosynthesis.This chelating reaction activate the metal and after that terminating the reaction. Crude enzyme preparations from the roots of pea, which mundanely contain both GSH and homo-GSH (g-Glu- Cys-b-Ala), could utilize GSH efficiently, but homo- GSH or g-Glu-Cys-Ser less efficiently, as substrates for PC synthesis 1995). In the presence of both GSH and homo-GSH, synthesis of homo-PCs was enhanced. These observations were interpreted to designate the enzyme had a g-Glu-Cys donor site that was relatively categorical for GSH but a less categorical acceptor site, able to utilize each of the three substrates. Little is kenned about the tissue specificity of PC synthase expression and/or PC biosynthesis. In the only study of tissue-concrete PC synthase expression to date, the activity was detected in the roots and stems of tomato plants but not in leaves or fruits (Chen et al,1997 and Rama Rao Karri, 2014).

The accumulation of a novel Cd-binding peptide:

Novel Cd-binding peptides (CdBPs), enhanced metal binding properties. A Cd-binding peptide, HSQKVK, isolated from a phage-displayed peptide library was narrated to increase the metal tolerance of transgenic tobacco plants when grown on media supplemented with 30 μM CdC. The best transgenic lines expressing CdBP fused in quadruplicate to the N-terminal of GFP, reached almost twice the biomass of the controls although they accumulated approximately the same amount of Cd.

Future prospects:

The uptake of metals by plants happens mainly through the roots. Plant root elements, such as root depth, penetration toward anaerobic areas and root weight are significant to principal in the period of plants favorable for phytoremediation. Also, the read of hypersensitive mutants, for lesson Arabidopsis thaliana mutants that exhibit hypersensitivity to Cd, Cu and Hg can give also information in the construal of the mechanisms that rule metal gathering. Numerous attempts to engineer the production of MTs in plants to increase metal tolerance and/or gathering bear been reported. Instantly a age, nevertheless, it is mainly the mannequin cultivate species that receive been genetically engineered. The engineering of crop plants or towering biomass plants would be a further step for the expansion of metal accumulating plants for phytoremediation. Indian mustard, a high biomass plant, as well as barley, has been proposed to have phytoremediation potential; in additament, transgenic poplar has recently been developed for Hg/As phytoremediation. Yellow poplar expressing a modified merA, relinguished ten times more basic Hg than untransformed plantlets; merA mutates the highly deadly Hg2+ to the ample less harmful, initial Hg. Moreover, arbitrary approaches such as DNA shuffling random priming recombination and staggered extension process can be acclimated to direct the evolution of enzymes or pathways involved in metal detoxification. Engineering whole pathways in plants is a promising approach of phytoremediation and there are possibilities of inserting multiple genes. The most significant advances are of PC biosynthesis and its function, have come from molecular genetic studies using a variety of model systems. These will perbetuate to provide a wealth of mutants for biochemical, molecular, and physiological analysis. Fascinating questions relating to the roles of PC synthase and PCs themselves in different organisms, possibly including animal species, remain to be answered. The isolation of PC synthase genes from a number of species will sanction a considerably preponderant understanding of the mechanism of metal activation of PC biosynthesis and the catalytic mechanism itself. There is considerable potential for the application of that understanding to optimizing the process of phytoremediation.

CONCLUSIONS

Plants appear to contain a diversity of metal-binding metallothioneins with the potential to perform distinct roles in the metabolism of different metal ions. The heterologous

expression of native and/or genetically modified peptides and protein emerges to be an charming key to edify the metal-binding abilities of these microbes. In order to restore environmental balance the bioremediation technique evidently does indicate several benefits and is one of the most preferred methods to deal with restoration of environment. Though improvement of plants by genetic engineering opens up new possibilities for phytoremediation, it is still in its research and development phase, with many technical issues needing to be addressed. Nevertheless, the described samples are placid mainly tested at a laboratory order and It continues to be seen whether bioremediation be an industrially adequate opinion.

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REFERENCES

1. Berka, T., Shatzman, A., Zimmerman, J., Strickler, J. and Rosenberg, M., 1988. Efficient expression of the yeast metallothionein gene in Escherichia coli. Journal of Bacteriology. 170(1): 21–26.
2. Broeks, A., Gerrard, B., Allikmets, R., Dean, M. and Plasterk R.H. 1996. Homologues of the human multidrug resistance genes MRP and MDR contribute to heavy metal resistance in the soil nematode Caenorhabditis elegans. European Molecular Biology Organization Journal. 15:6132–6143.
3. Chen,J.and Goldsbrough, P.B. 1994. Increased activity of g-glutamylcysteine synthetase in tomato cells selected for cadmium tolerance. Plant Physiology.106: 233–239.
4. Chen, J., Zhou, J., Goldsbrough, P.B., 1997. Characterization of phytochelatin synthase from tomato. Plant Physiology. 101: 165–172.
5. Clemens, S., Kim, E.J., Neumann, D. and Schroeder, J.I. 1999. Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. European Molecular Biology Organization Journal. 18: 3325–3333.
6. Cobbett, C.S., May, M.J., Howden, R. and Rolls, B., 1998. The glutathione-deficient, cadmium-sensitive mutant, cad2-1, of Arabidopsis thaliana is deficient in g glutamylcysteine synthetase. Plant Journal. 16:73–78.
7. Dameron, C.T., Reese, R.N., Mehra, R.K., Kortan, A.R., Carroll, P.J., Steigerwald, M L., Brus, L.E. and Winge, D.R. 1989. Biosynthesis of cadmium sulphide quantum semiconductor crystallites. Nature. 338: 596–597.
8. Dorhalc, F.D., Elmayan, T., Deroton, C.H., Tepfer, M. and Dehys, L., 1998.Cadmium partitioning in transgenic tobacco plants expressing a mammalian metallothionein gene. Molecular Breeding. 4:83–90.
9. Delhaize, E.P. and Ryan, R., 1995. Aluminum toxicity and tolerance in plants. Plant Physiology.107: 315–321.
10. Elmayan, T., and Tepfer, M., 1994. Synthesis of a bifunctional metallothionein β-glucuronidase fusion protein in transgenic tobacco plants as a mass of reducing leaf cadmium levels. Plant Journal. 6: 433-440.
11. Fordham-Skelton, A.P., Robinson, N.J. and Goldsbrough, P.B., 1998. Metallothionein-like genes and phytochelatins in higher plants. In S Silver, W Walden, eds, Metal Ions in Gene Regulation. Chapman & Hall London. pp 398–431.
12. Grill, E., Loffler, S., Winnacker, E.L. and Zenk, M.H., 1989. Phytochelatins, the heavy-metal-binding peptides of plants, are synthesized from glutathione by a specific g-glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase). Proceeding of the National Academy of Sciences USA.86: 6838–6842.
13. Hasegawa, M., Crowther, R.A., Jakes, R. and Goedert, M., 1997. Alzheimer-like changes in microtubule-associated protein Tau induced by sulfated glycosaminoglycans. Inhibition of microtubule binding, stimulation of phosphorylation, and filament assembly depend on the degree of sulfation.

Journal of Biological Chemistry. 272(52):33118-33124.
14. Hou, Y.M., Kim, R. and Kim, S., 1988. Expression of the mouse metallothionein-I gene in Escherichia coli: increased tolerance to heavy metals. Biochim et Biophys Acta. 951: 230–234.
15. Howden, R., Goldsbrough, P.B., Anderson, C.R. and Cobbett, C.S., 1995. Cadmium-sensitive, cad1 mutant of Arabidopsis thaliana are phytochelatin deficient. Plant Physiology. 107: 1059-1066.
16. Hoekema, A., Hirsch, P.R., Hooykaas, P.J.J. and Schilperoort, R.A., 1983. A binary plant vector strategy based on separation of vir- and T-region of the Agrobacterium tumefaciensTi-plasmid. Nature. 303: 179–180.
17. Howard, E.A., Zupan, J.R., Citovsky, V. and Zambryski, P.C., 1992. The VirD2 protein of A. tumefaciens contains a C-terminal bipartite nuclear localization signal: implications for nuclear uptake of DNA in plant cells. Cell. 68: 109–118.
18. Hunter, T.C. and Mehra, R.K., 1998. A role for HEM2 in cadmium tolerance. Journal of Inorganic Biochemistry. 69(4): 293-303.
19. Jacobs, F.A., Brousseau, R., Romeyer, F.M. and Beauchemin, M., 1989. Human metallothionein- II is synthesized as a stable membrane-localized fusion protein in Escherichia coli. Gene. 83: 95–103.
20. Kemper, M.A., Urrutia, M.M., Beveridge, T.J., Koch, A.L. and Doyle R.J. 1993. Proton motive force may regulate cell-wall associated enzymes of Bacillus subtilis. Journal of Bacteriology. 175: 5690-5696.
21. Klapheck. S., Schlunz, S. and Bergmann, L. 1995. Synthesis of phytochelatins and homo-phytochelatins in Pisum sativum L. Plant Physiology. 107: 515–521.
22. Kramer, U., Cotter-Howells, J.D., Charnock., J.M., Baker, A.J.M. and Smith, J.A.C., 1996. Free histidine as a metal chelator in plants that accumulate nickel. Nature. 379:635–638.
23. Kotrba, P., Pospisil, P., De Lorenzo, V. and Ruml, T., 1999. Enhanced metallosorption of Escherichia coli cells due to surface display of beta- and alpha-domains of mammalian metallothionein as a fusion to LamB protein. Journal of Receptor and Signal Transduction Research. 19:703–715.
24. Lovley, D.R. and Coates J.D., 1997. Bioremediation of metal contamination. Current Opinion in Biotechnology. 8: 285–289.
25. Larsen, P.B., Degenhardt, J., Stenzler, L.M., Howell, S.H. and Kochian L.V., 1998. Aluminum-resistant Arabidopsis mutant that exhibit altered patterns of aluminum accumulation and organic acid release from roots. Plant Physiology. 117: 9–18.
26. Murooka, Y. and Nagaoka, T. 1987. Expression of cloned monkey metallothionein in Escherichia coli. Applied and Environmental Microbiology. 53: 204–207.
27. Maiti, I.B., Wagner, G.J. and Hunt, A.G., 1991. Light inducible and tissue specific expression of a chimeric mouse metallothionein cDNA gene in tobacco. Plant Sciences .76: 99–107.
28. Mejáre, M., Ljung, S. and Bülow, L., 1998. Selection of cadmium specific hexapeptides and their expression as OmpA fusion proteins in Escherichia coli. Protein Engineering. 11: 489–494.
29. Mehra, R.K., Tran, K., Scott, G.W., Mulchandani, P. and Sani, S.S., 1996. Ag (I)-binding to Rauser W.E., 1999. Structure and function of metal chelators produced by plants: the case for organic acids, amino acids, phytin and metallothioneins. Cell Biochemistry and Biophysics. 31: 19–48.
30. Mauro, J.M. and Pazirndeh, M., 2000. Construction and expression of functional multidomain polypeptide in Escherichia coli expression of the Neurospora crassa metallothionein gene. Letters in Applied Microbiology. 30:161-166.
31. Nedkovska, M. and Atanassov, A.I., 1998. Metallothionein genes and expression for heavym metal resistance. Biotechnology. 12: 11–16.
32. Odawara, F., Massaki, K., Suzuki, M., Oikawa, S., Emoto, T., Yamasaki, F. and Arias, A., 1995. Expression of human metallothionein-2 in Escherichia coli:

Cadmium tolerance of transformed cells. Journal of Biochemistry. 118: 1131–1137.
33. Pazirandeh, M., Bridget, M. and Rebeceae, L., 1998. Development of Bacterium –Based biosorbents: Enhance uptake of Cd and Hg by E.Coli expressing a metal binding motif. Applied and Environmental Microbiology. 64(10):4068-4072.
34. Rama rao Karri. 2014. Comparative treatment for paranychia infection with locally available medicinal plants and synthetic drugs of bacterial and fungal microorganisms. Biolife, 2(1), 282-290.
35. Raskin, I., Nanda Kumar, P.B.A., Dushenkov, S. and Salt, D.E., 1994. Bioconcentration of heavy metals by plants. Current Opinion in Biotechnology. 5:285–290.
36. Silver, S., 1992. Bacterial heavy metal detoxification and resistance systems. In Biotechnology and Environmental Science: Molecular approaches (Mongkolsuk S., et al., eds), Plenum Press. pp. 109–129.
37. Sayers, Z., Broullion, P. and Michell, H., 1999. Expression of Saccharomyces cerevisiae copper-metallothionein gene in Escherichia coli and characterization of the recombinant protein. European Journal of Biochemistry. 212: 521–528.
38. Salt, D.E, and Rauser, W.E., 1995a. MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. Plant Physiology. 107:1293–1301.
39. Salt, D.E., Raskin, I., Robert, D.S., 1995b. Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Bio/Technology.13:468–474.
40. Silver S., 1996.Bacterial heavy metal resistance: New surprises. Annual Review of Microbiology. 50: 753–789.
41. Sousa,C., Cebolla, A. and De Lorenzo, V.,1996. Enhanced metalloadsorption of bacterial cells displaying poly His peptides. Bio/Technology. 14: 1017–1020.
42. Stefanov, I., Frank, J., Gedamu, L. and Misra, S., 1997. Effect of cadmium treatment on the expression of chimeric genes in transgenic seedlings and calli. Plant Cell Reports.16: 291–294.
43. Valls, A.M., Gonzàlez-Duarte, R., Atrian, S. and De Lorenzo, V., 1998. Bioaccumulation of heavy metals with protein fusions of metallothionein to bacterial OMPs. Biochemie. 80: 855–861.
44. Wagner, G.J., 1993. Accumulation of cadmium in crop plants and its consequences to human health. Advance Agronomy. 51:173–212.
45. Xiang, C. and Oliver, D.J., 1998. Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in Arabidopsis. Plant Cell. 10: 1539–1550.
46. Yong, L.Z., Pilon-Smits, E.A.H., Tarun, A.S., Weber, S.U., Jouanin, L. and Terry, N., 1999. Cadmium tolerance and accumulation in Indian mustard is enhanced by over expressing g-glutamylcysteine synthetase. Plant Physiology. 121: 1169–1177.
47. Zenk, M.H., 1996. Heavy metal detoxification in higher plants: a review. Gene. 179: 21–30.
48. Zhu, Y.L., Pilon-Smits, E.A.H., Jouanin, L. and Terry, N., 1999. Overexpression of glutathione synthetase in Indian mustard enhances cadmium accumulation and tolerance. Plant Physiology. 119: 73–79.

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