Project Number: 55118   Title: “Plant Rhizosphere Effects on Metal Mobilization and Transport

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DOE Problems Addressed: This project addresses phytoremediation of subsurface metal contamination. We are generating fundamental knowledges on how plants mobilize and sequester heavy metals from soils. This information should be applied to facilitating the design of both phytoextraction and stabilization of heavy metals in the subsurface zone.

RESEARCH OBJECTIVE

This project seeks to obtain a mechanistic understanding of mobilization and sequestratoin of heavy metal ions by plants via root exudation.

Research Progress and Implications

As of Feb, 2000, our work has focused on examining the influence of important rhizosphere factors (i.e. root exudation chemistry and soil organic matter) on metal ion sequestration and plant metabolism in crop plants such as barley, wheat, and rice. In order to understand the chemistry involved in metal ion mobilization by plants, we developed advanced NMR methodology in combination with GC-MS for a comprehensive profiling of root exudates[1], which has been a difficult challenge by conventional approach. Using a similar approach, the intracellular profiles of metal reactive compounds (MRC) in plants are also acquired, which is important to the understanding of how metal ions are sequestered within plants. Moreover, we have completed the development of a convenient gel electrophoretic method for characterizing tissue thiol-rich peptides (important to sequestration of Cd and other pollutant metals). These methodologies have been applied to investigate the relationship between MRC production and metal ion sequestration as a function of plant species, wheat genotypes, transition metal and Cd treatments, as well as soil organic matter (SOM). Experiments had the emphasis on wheat since a full set (7) of Chinese Spring (CS) genotypes (each with one chromosome addition from the salt and Al-tolerant European saltgrass, *Lophopyrum* sp.) are available from Dr. J. Dvorak, UC Davis. Utilizing these and other recombinant wheat genotypes, functional genomic maps are now being generated by Dr. Dvorak as part of an NSF-funded functional genomic center.

The root exudate profiles differed both qualitatively and quantitatively among barley, CS wheat, and rice plants (Figure 1). Under Fe deficiency, the family of mugineic acid (MA) phytosiderophores (PS) were among major components in barley and wheat exudates but was below NMR detection in rice exudate. The barley and wheat exudates were also generally higher in MRC (e.g. amino and organic acids) concentrations. These differences may be related to a better ability of barley and wheat to extract Fe than rice plant.

The increase in exudation of mugineic acids (MAs) under Fe deficiency in barley (3-*epi*-hydroxy-MA, MA, and 2’-DMA) and CS wheat (2’-deoxymugineic acid, 2’-DMA) is consistent with their role in Fe acquisition. The MAs may also be involved in the sequestration of Cu, Zn, and Mn. However, the MAs exudation were not correlated with Cd acquisition by either barley or wheat plants. This is contrary to the general notion that PS may mediate heavy metal uptake by plants. In addition, the Cd treatment caused a large increase in the sequestration of transition metals into wheat, which was not related to root MRC exudation. This Cd effect is not generally known and may be due to a disrupting effect of Cd on root membrane. It is also possible that some MRC may inhibit rather than enhance metal ion uptake, then the Cd-elicited decrease in exudation may alleviate this inhibition, and results in increased metal sequestration into plant tissues.

In addition to root exudation, the role of SOM (e,g, humics, a prominent class of soil MRC) in metal ion sequestration has been investigated. This information is grossly lacking but necessary for understanding metal ion mobilization and sequestration in the soil environment. The analysis to date indicates that humate plus Cd treatment of CS wheat plants greatly enhanced sequestration of transition metals in roots. This result is contrary to the expected role of humates as a competitive chelator for metals ions. It is likely that soil humates may directly mediate metal ion uptake via their cotransport with metal ions into wheat roots. This is supported by the stimulating effects of humate on MRC metabolism in wheat plants.

We have also begun to characterize the 8 wheat genotypes plus the CS parent for their difference in capacity of metal sequestration and root exudation profile. Analysis to date indicates that the genotypic profile of Cd sequestration differed from those of the transition metals and that the metal sequestration system in wheat may reside on at least two chromosomal loci[2].

The MRC involved in intracellular sequestration of Cd(II) was investigated using the recently developed method that combined fluorescent tagging of thiol-rich peptides by bromobimane with SDS-PAGE. This method allowed a fast and simultaneous assay of both phytochelatins (PC) and metallothionein (MT)-like proteins, as illustrated for wheat, rice, and clam in Figure 2. For both wheat and rice, Cd treatments induced a large accumulation of PC (<3.5 kD SH-rich peptides) but not MT in root and shoot. The PC accumulation was sensitive to Cd sequestration only. These PC appeared to have high affinity for Cd and Pb, as revealed by capillary electrophoresis coupled with ICP-MS. These results suggests PC's role in sequestering heavy metals, particularly in roots where much higher amounts of Cd were sequestered than in shoots.

In conclusion, our results indicate that both non-peptidic MRC and SH-rich peptides may be important to heavy metal sequestration in plant tissues. They may all contribute towards plant's ability to tolerate high levels of metal accumulation and govern subsequent fate of metals in the soil environment. In addition, plant roots sequestered a high amount of metals, which will not be practical to remove from soils by harvest. From the phytoremediation standpoint, the long-term fate of these metals including bioavailability and leachability will need to be addressed. Depending on their speciation and interaction with soil organic matter and minerals, pollutant metals may be "entombed" with aging. Such aging process, if understood, may be enhanced to facilitate subsurface phytoremediation of metal contaminants.

Figure 1. ^{1}H NMR spectra of whole root exudates from barley, wheat, and rice under a combined Fe deficient and Cd treatment. GAB, γ-amino-butyrate; MES, morpholino-ethanesulfonate; *epi*-OHMA, *epi*-hydroxy mugineic acid; 2'-DMA, 2'-deoxy-mugineic acid; Unk polyol, unknown

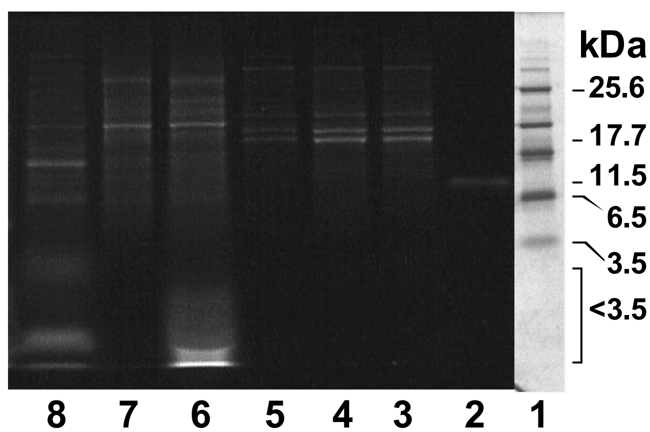

Figure 2. SDS-PAGE of bromobimane-tagged SH-rich peptides and proteins from wheat, rice, and clam tissues. Lanes 1, MW standards; 2, rabbit metallothionein (MT); 3-5, clam MT extracts; 6,7, Cd-treated and control wheat root extracts; 8, Cd-treated rice shoots, respectively.

Fan, T.W.-M., J. Pedler, A.N. Lane, D. Crowley, and R.M. Higashi, Comprehensive Analysis of Organic Ligands in Whole Root Exudates Using NMR and GC-MS, *Analytical Biochemistry* 251, 57-68 (1997)

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