Genetic and Molecular Dissection of Arsenic Hyperaccumulation in the fern Pteris vittata.

The fern *Pteris vittata* (Pteridaceae) is extraordinary, as it and its close relatives are the only known organisms to tolerate and accumulate up to 2% of their tissue dry weight as arsenic, which is 100 to 1000-times more arsenic than other organisms are able to tolerate. This trait makes it an excellent model system among plants, animal and microbes for the study of arsenic metabolism, accumulation and resistance mechanisms. The goals of the proposed research were to identify genes involved in arsenic metabolism and resistance in this fern. We succeeded in cloning two genes from *P. vittata*, named *PvACR2* and *PvACR3*, that encode an arsenate reductase and a putative arsenite effluxer protein, respectively. PvACR2 has arsenate reductase activity *in vitro* and complements the arsenate reductase activity of ACR2 in a yeast *Δacr3* mutant (Ellis et al., 2006). The *P. vittata PvACR3* gene complements the arsenite efflux function of *ACR3* in a yeast *Δacr3* mutant, and although its function in *P. vittata* is currently unknown, we believe that it is involved in sequestering arsenite within the vacuole following the reduction of arsenate to arsenite by PvACR2.

Another goal of the proposed research was to study the physiology of arsenic uptake and hyperaccumulation in the *P. vittata* sporophyte. In comparing arsenic content in different parts of *P. vittata* plants grown in the presence of arsenic, our lab plus other labs demonstrated that the highest levels of arsenic occur in the fronds (Ma et al., 2001; Chen et al., 2002; Lombi et al., 2002; Tu et al., 2002; Tu and Ma, 2002) with up to 25 times more arsenic in the fronds than in the roots (Tu and Ma, 2002). These studies have also shown that the sporophyte efficiently takes up arsenate As(V) from the soil and rapidly transports it to the shoot in the xylem, mainly as arsenate (Chen et al., 2005), where it arrives in the petiole and midrib of the frond as arsenate (Hokura et al., 2006; Pickering, 2006). Within the frond, arsenic is localized to upper and lower epidermal cells and trichomes where it is likely stored in the vacuoles of these cells (Lombi et al., 2002; Li et al., 2005). Over 95% of arsenic is stored in the fronds as free arsenite As(III), as determined by X-ray absorption spectroscopy (XAS) (Lombi et al., 2002; Webb et al., 2003; Ze-Chun et al., 2004; Pickering, 2006) and high pressure liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS) (Wang et al., 2002; Zhao et al., 2003). These results suggest that the arsenate that is taken up by the root is transported by the vascular tissue to the frond, and reduced to arsenite as it is compartmentalized for storage in the lamina of the frond.

Although the majority of arsenic in the fronds accumulates as arsenite (As(III)), only a very minor portion (<5%) of As(III) is coordinated by thiol groups in *P. vittata* (Lombi et al., 2002; Wang et al., 2002; Zhang et al., 2002; Webb et al., 2003; Zhao et al., 2003; Pickering, 2006; Singh and Ma, 2006) and in *P. cretica* (Raab et al., 2004). By directly visualizing arsenic in the sporophyte, we found that this minor thiolate coordination of As(III) in the *P. vittata* sporophyte is limited to a cylindrical sheath, 40-50 μm thick, immediately surrounding arsenate in the veins of the vascular tissue of the fronds (Pickering, 2006). This observation indicates that thiolate coordination may be involved in the reduction of arsenate as arsenic is transported from the vascular tissue of the frond and ultimately stored in cells of the lamina. If this thiol coordination is a necessary step in the reduction of arsenate, arsenite quickly loses its thiol coordination as it is transported to and stored in cells of the lamina of the frond.

The data generated from this research will be used to test whether other plants can be genetically modified to tolerate and hyperaccumulate arsenic. If this trait can be transferred to other plants, they can then be used as an effective and efficient means to remove arsenic from comtaminated soils.

References Cited:

Chen, T., Wei, C., Huang, Z., Huang, Q., Lu, Q., and Fan, Z. (2002). Arsenic hyperaccumulator *Pteris Vittata* L. and its arsenic accumulation. Chinese Science Bulletin.

Chen, T., Huang, Z., Huang, Y., and Lei, M. (2005). Distributions of arsenic and essential elements in pinna of arsenic hyperaccumulator Pteris vittata L. Science in China **48,** 18-24.

Ellis, D.R., Gumaelius, L., Indriolo, E., Salt, D., and Banks, J.A. (2006). A novel arsenate reductase from the arsenic hyperaccumulating fern Pteris vittata. Plant physiology **141,** 1544-1554.

Hokura, A., Omuma, R., Yasuko, T., Kitajima, N., Abe, T., Salto, H.Y., Hy, S., and Nakai, I. (2006). Arsenic distribution and speciationin an arsenic hyperaccumulator fern by X-ray spectrometry utilizing a synchrotron radiation source. J Anal Atomic Spectrometry **21,** 321–328.

Lombi, E., Zhao, F.-J., Fuhrman, M., Ma, L., and McGrath, S.P. (2002). Arsenic distribution and speciation in the fronds of the hyperaccumulator *Pteris vittata*. New Phytologist **156**.

Ma, L.Q., Komar, K.M., Tu, C., Zhang, W., Cai, Y., and Kennelley, E.D. (2001). A fern that hyperaccumulates arsenic. Nature **409,** 579.

Pickering, I., L Gumaelius, HH Harris, RC Prince, G Hirsch, JA Banks, DE Salt, GN George. (2006). Localizing the Chemical Transformation of Arsenate in a Hyperaccumulating Fern. Environ. Ci. Technol. 40:5010-5014.

Raab, A., Feldman, J., and Meharg, A.A. (2004). The Nature of Arsenic-Phytochelatin Complexes in *Holcus lanatus* and *Pteris cretica*. Plant physiology **134,** 1113-1122.

Singh, N., and Ma, L.Q. (2006). Arsenic speciation, and arsenic and phosphate distribution in arsenic hyperaccumulator Pteris vittata L. and non-hyperaccumulator Pteris ensiformis L. Environ Pollut **141,** 238-246.

Tu, C., and Ma, L.Q. (2002). Effects of Arsenic Concentrations and Forms on Arsenic Uptake by the Hyperaccumulator Ladder Brake. Journal of Environmental Quality **31,** 641-647.

Tu, C., Ma, L., and Bondada, B. (2002). Arsenic accumulation in the hyperaccumulator Chinese Brake and its utilization potential for phytoremediation. Journal of Environmental Quality **31,** 1671-1675.

Wang, J., Zhao, F.-J., Meharg, A.A., Raab, A., Feldman, J., and McGrath, S.P. (2002). Mechanisms of Arsenic Hyperaccumulation in Pteris vittata. Uptake Kinetics, Interactions with Phosphate, and Arsenic Speciation. Plant physiology **130,** 1552-1561.

Webb, S.M., Gaillard, J.F., Ma, L., and Tu, C. (2003). XAS Speciation of arsenic in a hyperaccumulating fern. Environmental Science and Technology **37,** 754-760.

Zhao, F.J., Wang, J.R., Barker, J.H.A., Schat, H., Bleeker, P.M., and McGrath, S.P. (2003). The role of phytochelatins in arsenic tolerance in the hyperaccumulator Pteris vittata. New Phytologist **159,** 403-410.

STI Deliverables:

Journal articles resulting from this award:

Ellis, D.R., Gumaelius, L., Indriolo, E., Salt, D., and Banks, J.A. (2006). A novel arsenate reductase from the arsenic hyperaccumulating fern Pteris vittata. Plant physiology **141,** 1544-1554.

Gumaelius, L., Lahner, B., Salt, D., and Banks, J.A. (2004). Arsenic hyperaccumulation in gametophytes of *Pteris vittata*: A new model system for analysis of arsenic hyperaccumulation. Plant physiology **136,** 3198-3208.

Pickering, I., L Gumaelius, HH Harris, RC Prince, G Hirsch, JA Banks, DE Salt, GN George. (2006). Localizing the Chemical Transformation of Arsenate in a Hyperaccumulating Fern. Environ. Ci. Technol. 40:5010-5014.

Conference Proceedings:

Indriolo, E., Salt, D. and Banks, JA. (2007). Characterization of PvACR3 from Pteris vittata. Poster presentation at the American Society of Plant Biologists annual meeting, Chicago, Illinois.

Human resources:

The proposed research was conducted by Drs. Banks and Salt plue the following postdocs and students at Purdue University, who received extensive training in molecular, biochemical and genetic methods.

Dr. Danielle Ellis, postdoctoral research fellow
Dr. Luke Gumaelius, doctoral student, received PhD December 2006, thesis title: "Molecular Basis of Arsenic Hyperaccumulation in the Fern *Pteris vittata*"
Dr. Emily Indriolo, PhD student, will graduate December 2009.
Ms. Grace DeLay, undergraduate student