

Figure 1. *S. oneidensis* MR1 reduces soluble and solid-phase iron and manganese oxides and soluble uranium to insoluble oxides using specialized attachment proteins and components of the electron transport chain (illustrated by the cell membrane model on the left). The sketch on the right is based on tomography revealing UO_2 crystals formed on the cell's surface after reduction of uranium by *S. oneidensis* (courtesy of the National Center for Microscopy and Imaging Research, University of California, San Diego, and the Pacific Northwest National Laboratory).

its energetics, and what impact this has had on Earth's geochemistry.

Because of the importance of understanding metal reduction for bioremediation, the US Department of Energy (DOE; Germantown, MD) under its Genomes to Life program has brought together a diverse team of experts in physiology, ecology, genomics, proteomics, protein interactions, advanced cell imaging, regulatory control, metabolic modeling, and geochemistry to comprehensively study *S. oneidensis*. This group, known as the Shewanella Federation⁶ and led by James Fredrickson at DOE's Pacific Northwest National Laboratory, involves 14 institutions.

Although the Heidelberg paper focuses on *S. oneidensis*, genome-based analysis of other microbes is underway with a view to exploiting their potential uses in environmental biotechnology (for further information and sequencing status of these strains, see refs 7,8). Other metal-transforming strains whose genomes have been sequenced include two anaerobes, *Geobacter sulfurreducens* and *G. metallireducens*, and an aerobe, *Ralstonia metallidurans*. Although not a metal reducer, *Deinococcus radiodurans* has the notable ability to withstand very high doses of radiation by correctly re-assembling its genome after radiation-induced fragmentation⁹, a process currently being investigated through genomic analysis. The hope is to use this microbe to carry bioremediation traits into highly radioactive sites.

Where *Shewanella* and *Geobacter* species can use metals as electron acceptors, other microbes can use halogenated organic compounds for this function and in the process degrade halogenated pollutants. Genome sequences are already available for two microbes that grow by reductively dechlorinating chloroethenes: *Dehalococcoides* (an unusual Gram-negative bacterium) and *Desulfotibacterium* (a spore-forming Gram-positive bacterium). These sequences contain a large number of dehalogenases and

dehalogenase-like genes, most of whose functions are unknown. Exploiting this information in the engineering of new strains offers considerable promise for bioremediation research, as the chlorinated ethenes represent probably the most widespread, serious class of groundwater pollutants in the United States.

Finally, the genomes of several aerobic pollutant-degrading organisms have been sequenced. *Burkholderia* strain LB400, the most effective PCB degrader known, and *Rhodococcus* strain RHA1, a versatile Gram-positive PCB degrader, are being sequenced. As microbes cannot grow on PCBs, genomic information will indicate approaches that may ultimately overcome the metabolic blocks to growth. The genomes of *Pseudomonas putida* and *P. fluorescens*, two organisms that show considerable versatility in using aromatic hydrocar-

bons as their carbon source and thereby mineralizing these pollutants, have also been sequenced. In all these cases, considerable challenges exist in using these organisms or their processes in bioremediation. Understanding and improving expression, recruiting missing or improved enzymatic activities, enhancing bioavailability, and improving cell transport and survival are research goals that will be furthered by the availability of increasing amounts of genomic information.

1. Heidelberg, J.F. *et al.* *Nat. Biotechnol.* **20**, 1118–1123 (2002).
2. Nealson, K.H. & Saffarini, D. *Annu. Rev. Microbiol.* **48**, 311–343 (1994).
3. Lovley D.R. *et al.* *Arch. Microbiol.* **159**, 336–344 (1993).
4. Lloyd, J.R. & Lovley, D.R. *Curr. Opin. Biotechnol.* **12**, 248–253 (2001).
5. Lower, S.K., Hochella, M.F., & Beveridge, T.J. *Science* **292**, 1360–1363 (2001).
6. <http://www.shewanella.org>
7. <http://www.tigr.org>
8. <http://www.jgi.doe.gov>
9. Makarova, K.S. *et al.* *Microbiol. Mol. Biol. Rev.* **65**, 44–79 (2001).

Pumping out the arsenic

***Arabidopsis thaliana* has been engineered to contain two bacterial enzymes to help it remove arsenic from contaminated soils.**

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The toxicity of arsenic will be highly familiar to readers of detective stories, in which it commonly appears as the cause of death. More recently, arsenic has been shown to be a cause of liver, lung, kidney, and bladder cancer; in fact, population cancer risks from arsenic exposure may be comparable

to those from environmental tobacco smoke and radon in homes¹. Creating a better “pump” for removing arsenic from contaminated soils is thus an important goal in a world facing severe problems with arsenic pollution. In this issue, Dhankher *et al.*² have made some important strides forward by genetically engineering plants to remove arsenic more efficiently from soil. By overexpressing two bacterial genes in the small weed *Arabidopsis thaliana*, they have substantially increased the accumulation of arsenic in leaves—a necessary prerequisite to the use of phytoremediation

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(bioremediation mediated by plants) as an effective tool in arsenic cleanup.

Arsenic occurs naturally in rocks and soils and in water in contact with them; in other cases sites are polluted through agricultural practices (such as the use of arsenical pesticides), mining, and other industrial activities. Human exposure to arsenic through contact with soils and sediments is of great concern, as is the migration of arsenic through soils to ground water. The buildup of arsenic in groundwater is a serious problem in many areas of the world, notably Bangladesh, where over 40 million people drink well water containing toxic levels of arsenic³.

Most remediation strategies, such as soil removal and burial, are expensive and relatively inefficient. The use of plants to extract arsenic from soil ("phytoextraction") could be a cost-effective alternative. Separating arsenic from soil is difficult because arsenic is strongly retained by the soil, especially in oxidizing environments and at low pH. Even after its entry into the roots, arsenic is not readily transported to the leaves of most plants (Fig. 1). This is illustrated by the work of Pickering *et al.*⁴, who found that large amounts of arsenic were locked up in plant roots and that only relatively small amounts of arsenic (as arsenate and arsenite) were exported via the xylem to aboveground tissues. To make the phytoextraction of arsenic more effective, it is essential to find means to enhance the transport of arsenic from the root system to stems and leaves, where the metal can be easily harvested.

Dhankher *et al.* tackled this problem by overexpressing two bacterial genes in *A. thaliana*: one encoding arsenate reductase (*arsC*) and the other encoding γ -glutamylcysteine synthetase (γ -ECS) (Fig. 1). *ArsC* catalyzes the reduction of arsenate to arsenite in the stem and leaves. γ -ECS, which catalyzes the first step in the phytochelatin synthesis pathway, increases the pool of thiol compounds, including the polypeptide phytochelatin, throughout the plant. Thiol compounds detoxify arsenite by forming arsenic-protein thiolates, which can then be stored, probably in the vacuole (Fig. 1). This enables arsenic to accumulate at greater amounts in the leaves of the transgenic plants than in the wild type.

Boosting concentrations of thiol compounds to increase plant tolerance to toxic trace elements is not a new idea: it has been done by several research groups, including our own. We have overexpressed γ -ECS in Indian mustard (*Brassica juncea*) and found that these plants were more tolerant to arsenic (N.T., unpublished data) as well

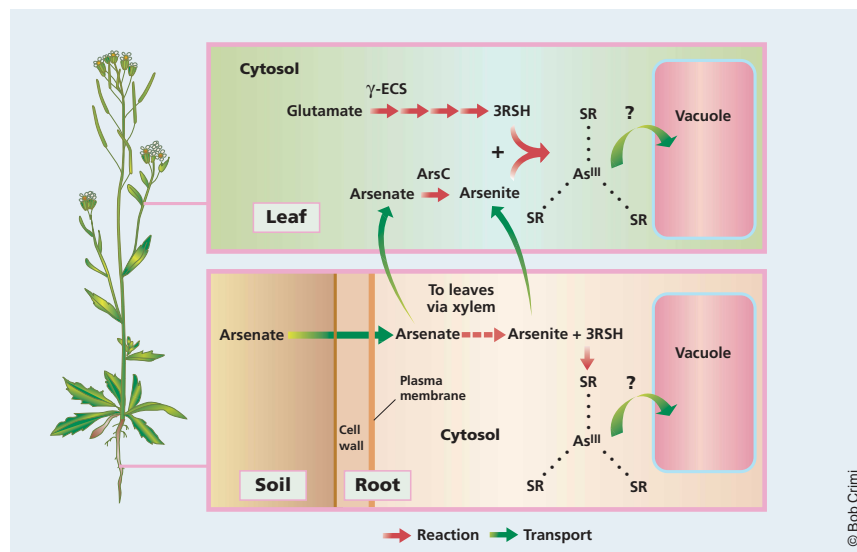


Figure 1. Uptake, transport, and metabolism in transgenic *A. thaliana* plants overexpressing two bacterial genes, *arsC* and γ -ECS. Arsenic in soil is found primarily in the oxidized form, arsenate (AsO_4^{3-}). A portion of the arsenate taken up into plant roots may be held in cell walls (possibly as FeAsO_4). The remainder crosses root membranes probably via transporters facilitating the uptake of phosphate⁴, which is chemically similar to arsenate. Inside the root cells, some arsenate is reduced to arsenite (AsO_3^{3-}). A small fraction of the arsenate and arsenite move from root to leaf via the xylem⁴. Arsenate is then reduced by the enzyme *ArsC* to arsenite, which combines with thiol compounds to form arsenic-thiolates. In this form, arsenic is probably shuttled irreversibly into the vacuoles, where it can no longer damage the plant.

as to cadmium⁵. What is new and significant about the work of Dhankher *et al.* is the tissue-specific expression of *arsC* in combination with the constitutive expression of γ -ECS. By expressing *arsC* under the control of a light-inducible promoter, the authors increased arsenate reduction only in the above-ground tissue and not in roots, thereby avoiding diminution of the root pool of arsenate. Thus, arsenate in the transgenic plants could move from root to leaf, where it was trapped by the combined action of *arsC* and γ -ECS (Fig. 1).

Will these transgenic plants really be useful for phytoremediation? The strategy used by Dhankher *et al.* of combining thiol-compound increase with a leaf-specific reductase enhanced both arsenic tolerance and accumulation, as compared with those of wild-type plants. The doubly transgenic plants were much more tolerant of high arsenic concentrations (15 μg arsenic/g), their biomass being sixfold greater than that of the wild type. Arsenic concentrations in shoots of doubly transgenic plants grown on 9.4 μg arsenic/g were threefold greater than those of the wild type. However, in comparison with the staggeringly efficient brake fern, *Pteris vittata*⁶, the transgenic plants produced by Dhankher *et al.* are much less impressive.

P. vittata, a natural arsenic hyperaccumulator, can tolerate soil concentrations of 1,500 μg arsenate/g and can accumulate up

to 23,000 μg arsenic/g in its shoots (fronds). The striking difference between *P. vittata* and arsenic non-accumulators is the enormous transport of arsenic from roots to shoots in *P. vittata*. In most plants, only a small fraction of the arsenic taken up from soil by roots accumulates in the above-ground tissue (<20%)^{3,7}, whereas *P. vittata* accumulates up to 95% of the arsenic in above-ground tissue.

As a final note, it should be emphasized that the molecular mechanisms involved in arsenic uptake and transport by the brake fern are unknown. At present, this prevents the identification of genes that could be used to transform fast-growing, high-biomass phytoremediators, such as Indian mustard. In contrast, the work of Dhankher *et al.* uses a defined molecular mechanism and thereby advances the state of the art in developing strategies to genetically enhance arsenic uptake and accumulation.

- Smith *et al.* *Environ. Health Perspect.* **97**, 259–267 (1992).
- Dhankher *et al.* *Nat. Biotechnol.* **20**, 1140–1145 (2002).
- Smedley, P. & Kinniburgh D. *Appl. Geochem.* **17**, 517–568 (2002).
- Pickering *et al.* *Plant Physiol.* **122**, 1171–1177 (2000).
- Zhu *et al.* *Plant Physiol.* **121**, 1169–1177 (1999).
- Ma *et al.* *Nature* **409**, 579 (2001).
- Carbonell-Barrachina, A., Carbonell, B., & Beneyto, M. *J. Plant Nutrition* **17**, 1887–1903 (1994).