

# Phytoremediation: A clean transition from laboratory to marketplace?

George E. Boyajian and Laura H. Carreira

Decades of industrial growth have left an international legacy of soil and water contaminated with a combination of toxic and potentially carcinogenic organic compounds and heavy metals. With many of these sites abandoned and a remediation market in the United States and European Union alone expected to exceed \$20 billion annually, the demand for cost-effective cleanup solutions has soared. In recent years, the potential of plants for environmental cleanup—phytoremediation—has been recognized, and US government agencies and private corporations have responded by increasingly supporting research in this area.

Exaggerated claims of success in the

early years of bacterial remediation led to inflated expectations for the capabilities of bioremediation. Therefore, it is incumbent upon phytoremediation researchers to conduct basic laboratory work first, before attempting field implementation. In this context, the report in this issue by Goel et al.<sup>1</sup> both advances our basic knowledge of plant biochemistry and demonstrates that plants are indeed capable of tackling such exotic xenobiotic contaminants as nitroglycerin.

Using both plant cells and extracts, Goel and colleagues show that sugarbeet can degrade nitroglycerin (a nitrate ester) by intracellular processes, probably by a hydrolysis reaction. The novelty of the degradation process lies in the fact that the nitro groups on nitroglycerin are cleaved from an organic backbone. This mechanism contrasts with that of a nitroreductase we have characterized from sediment<sup>2</sup>, which requires ferredoxin and NADPH for the reduction of the nitrate group to an amine group on a ring


structure. Whatever the mechanism, these results (and others) suggest that plants are capable of remediating sites contaminated with organic nitrate esters, xenobiotics that were long thought to be recalcitrant to biodegradation.

Although the intracellular mechanisms of phytodegradation of organics have yet to be documented fully, the ability of plant-derived enzymes to degrade various compounds has been amply demonstrated<sup>3</sup> (see Table 1). Planting selected species (identified via immunoassays) containing the appropriate enzymes could be completed at a fraction of the cost of traditional engineering methods or used to accelerate cleanups already in progress. Phytoremediation has already been successfully implemented by the US Air Force to clean trichloroethylene from groundwater using poplar trees and by the US Army to clean 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) from contaminated wetlands

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Table 1. Plant enzymes and the wide range of contaminants they can degrade.\*

Plant enzyme	Status of development	Contaminants degraded
Nitroreductase <sup>2</sup>	Field tested	TNT, dinitromonoaminotoluene, and mononitrodiaminotoluene
	Plants grown on laboratory substrate	Nitrotoluene, dinitrotoluene, nitrophenols, dinitro phenols, trinitrophenols, nitrobenzenes, chlorinated nitrobenzenes, RDX
Laccase <sup>6</sup>	Field tested	Triaminotoluene
Dehalogenase <sup>7</sup>	Plants grown on laboratory substrate	Ethylene, methylene, hexachloroethane, perchloroethylene and its brominated analog, TCE, dichloroethylene, chloroform, and carbon tetrachloride
Nitrilase <sup>8</sup>	Plants grown on laboratory substrate	4-Chlorobenzonitrile

\*Source: PhytoWorks, Inc., 1996. Plant peroxidase has also been isolated, but not yet tested.

(using a variety of plants). In pilot sites at numerous industrial facilities for petroleum hydrocarbons and wood preservatives, phytoremediation with grasses (and their associated bacterial populations) has also had limited success.

The application of phytoremediation for the clean up of soil and water contaminated with toxic levels heavy metals is another area that has recently received attention. Certain plants translocate metals from their surroundings and accumulate them in their above-ground tissues at high concentrations so that they can be harvested and removed from the site. Two routes are currently being explored to develop metal-accumulating plants: Genetic engineering and the selective breeding of naturally occurring hyperaccumulators—plants that contain >1% wet weight in metal<sup>4</sup>.

By inserting an altered mercuric ion reductase gene (*merA*) into *Arabidopsis thaliana*<sup>5</sup>, a group headed by Richard Meagher at the University of Georgia (Athens) recently produced a mercury-resistant transgenic plant that volatilizes mercury into the atmosphere. Transgenic *merA* plants are soon to be tested in soil; results to date suggest that the cost of phytoremediation of mercury-contaminated soils will be one-tenth to one-hundredth the cost of other traditional engineering methods, including landfilling, thermal treatments, and chemical extraction.

While the mechanisms of metal uptake and sequestration by metal-accumulating plants are poorly understood, hyperaccumulation probably evolved in response to insect attack. Both hyperaccumulating species and related taxa are currently being tested and bred for phytoremediation potential. *Brassica* species show a propensity to accumulate a number of heavy metals, including lead (the most common metal

contaminant in Superfund sites). For example, Phytotech Inc. (Monmouth Junction, NJ) is developing a proprietary method to remove lead from soils, combining the use of hyperaccumulating *Brassica* strains and the addition of EDTA to soil (to solubilize bound lead making it available for root uptake). Lead-laden plants are then harvested and removed from the site.

From most perspectives, plants are ideal for environmental cleanup: Capital cost is low, ongoing operating costs are minimal, implementation is easy and noninvasive, and public acceptance is high. More relaxed US Environmental Protection Agency (EPA, Washington,

DC) standards regarding bioavailability and risk-based assessments make regulatory acceptance more likely. However, clean-up rates may prove slower than conventional methods, and although plants have upwards of 100 million miles of roots per acre, their root systems may not extend deeply enough to eliminate all of the contamination.

The next challenge facing phytoremediation in the marketplace is for scientists to work closely with environmental engineers and regulators to prove the technology's efficacy in pilot sites. However, with a sound footing in basic research and a new regulatory environment supportive of innovative technologies, phytoremediation is increasingly being viewed as a cost-effective and user-friendly alternative to traditional methods of environmental cleanup.

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## Carboxypeptidase E: A surprise player in protein sorting

James Kling

Cells either release proteins constitutively—a slow bleed from the rough endoplasmic reticulum (RER), where proteins are synthesized, to the cell membrane—or may follow the regulated secretory pathway (RSP), in which proteins are hoarded in granules until an external signal demands their sudden release. Because proteins are often processed into their active form in the granules, a kink in the system could result in release of inactive precursors. For example, before insulin emerges from islet cells to regulate blood sugar levels, its precursor—proinsulin—must be properly targeted to granules and processed by proteases. If proinsulin is instead released constitutively, hyperproinsu-

linemia will result, causing diabetes and potentially obesity.

But how a cell sorts the proteins has remained a mystery. In a 1985 article in *Science*, Regis Kelly of the University of California at San Francisco hormone research institute gave evidence that the RSP pathway is not the default pathway for protein secretion, as was previously supposed. Instead, Kelly believed that the RSP proteins are the exception, selected by some mechanism of the cell, whereas other secretory proteins slowly migrate—unguided—from the RER to the cellular membrane.

Kelly's assertion touched off a long search for the protein signal and cellular receptor that govern the RSP, and now a study by Y. Peng Loh and her colleagues at the National Institute of Child Health and Human Development (Bethesda, MD)—published in the

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