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Phytoremediation of small organic contaminants using transgenic plants

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Abstract

The efficacy of transgenic plants in the phytoremediation of small organic contaminants has been investigated. Two principal strategies have been pursued (1) the manipulation of phase I metabolic activity to enhance *in planta* degradation rates, or to impart novel metabolic activity, and (2) the enhanced secretion of reactive enzymes from roots leading to accelerated *ex planta* degradation of organic contaminants. A pair of dehalogenase genes from *Xanthobacter autotrophicus* was expressed in tobacco resulting in the dehalogenation of 1,2-dichloroethane, which was otherwise recalcitrant. A laccase gene from cotton was overexpressed in *Arabidopsis thaliana* resulting in increased secretory laccase activity and the enhanced resistance to trichlorophenol in soils. Although the results to date are promising, much of the work has been limited to laboratory settings; field demonstrations are needed.

Introduction

Low molecular weight organic contaminants include many that are of concern to human health and the environment. Chlorinated solvents such as carbon tetrachloride (CT), 1,1,1-trichloroethane (TCA), perchloroethylene (PCE), trichloroethylene (TCE), and chloroform have been widely used for decades resulting in inadvertent release and contamination of soil and groundwater [1,2]. Leakage from underground petroleum storage tanks has resulted in the contamination of water supplies by benzene. Chlorophenols are commonly used wood preservatives and biocides; trichlorophenol is associated with human health risks. Bisphenol A, a phenolic compound, is a plasticizer that has been widely used for over 50 years and is one of the most common pollutants in groundwater [3]. There is a continuing need to develop cost-effective remediation techniques.

The phytoremediation of organic contaminants has the potential to be a low-cost remediation method for contaminated soil and groundwater [4–9]. Phytoremediation may be substantially less costly than engineered methods (USEPA, URL: <http://clu-in.org/products/intern/phytot-ce.htm>). The potential value of biomass production, soil stabilization, and carbon sequestration could add to the economic viability of phytoremediation [10]. In less-developed regions of the world, resources may not be available for engineering—intensive methods, leaving phytoremediation as one of the few available options [11].

The potential benefits of phytoremediation are offset by uncertainty. The uncertainty related to the duration and effectiveness of cleanup may make phytoremediation economically unfavorable [12]. Cleanup time may be the most crucial measure of economic viability [13]. Accordingly, there is incentive to develop methods to optimize the rate and reliability of phytoremediation. Methods involving the genetic modification of plants have been reviewed previously [14–18]. Here we review recent developments in genetic modifications of plants for enhanced phytoremediation of low molecular weight organic contaminants (Figure 1).

Enhanced *in planta* metabolism

There are several mechanisms in the phytoremediation environment that may lead to enhanced removal of organic contaminants. *In planta* processes include uptake and diffusion through the roots, trunk, or leaves, sorption, and transformation and/or sequestration via tree metabolic activity. Alternatively, *ex situ* degradation may occur via enhanced microbial activity in the rhizosphere, or the excretion of proteins and cofactors resulting in non-specific activity. Uptake of non-ionic organics into plant roots is believed to be a diffusive process dependent largely on the octanol/water partition coefficient and Henry's law constant [19]. Aquaporins and aquaglyceroporins facilitate the selective transport of water and neutral solutes such as urea, glycerol, lactic acid, and ammonia by contributing to the permeability of plant membranes for these compounds [20,21]. They may provide an alternative route for the uptake of low molecular weight contaminants. *In planta* metabolic activity includes chemical modification or activation (phase I), conjugation (phase II), and transport/compartmentalization (phase III) [22].

Phase I transformations of xenobiotic organics includes oxidation, reduction, and hydrolysis reactions. Cytochrome P450 (P450)-mediated oxidation reactions are the most important [23]. P450s encompass the largest family of plant proteins; in humans, 11 P450 proteins catalyze 90% of reactions with xenobiotics [24]. Transgenic modifications performed to enhance phytoremediation of VOCs have largely focused on the expression of these P450s in plant systems. Isoform P450 2E1 (CYP2E1) has been identified in mammalian systems as important in the metabolism of several xenobiotic contaminants [25]. Tobacco plants (*Nicotiana tabacum* cv. *Xanthii*) were transformed to express human CYP2E1 under the Mac promoter resulting in a marked increase in metabolism of TCE and ethylene dibromide compared to vector controls in hydroponic reactors [26]. Trichloroethylene was oxidized mainly to trichloroethanol, which was further transformed to conjugated trichloroethanol-glucoside [27]. The transformed tobaccos also metabolized vinyl chloride (VC), CT, benzene, toluene, chloroform, and bromodichloromethane, but not PCE or TCA [28].

Hybrid poplar (*Populus tremula* × *Populus alba*) were transformed with rabbit CYP2E1 under the cauliflower mosaic virus (CaMV) 35S promoter to take advantage of increased metabolism in a deep-rooted, fast-growing tree species. Transformed poplar hairy root cultures exposed to TCE resulted in a higher production of chloral and trichloroethanol than controls [29]. Cuttings were used in a series of laboratory experiments that demonstrated more-rapid uptake of TCE, VC, CT, chloroform, and benzene from hydroponics and TCE and benzene from the air [30•]. The rate of uptake from the air was significantly lower than from hydroponics, suggesting possible mass-transfer limitations. This was the first report of transgenic trees being utilized to enhance the uptake of such a wide variety of environmentally important chemicals.

The introduction of genes coding for degradation of organics into plants has generally been restricted to single step transformations. The exception is the introduction of two genes for the degradation of 1,2-dichloroethane (1,2-DCA) into *Arabidopsis* [31,32••]. The soil bacteria *Xanthobacter autotrophicus* GJ10 catalyses the degradation of 1,2-DCA through the

toxic intermediates 2-chloroethanol, 2-chloroacetaldehyde, chloroacetic acid, to glycolate [31]. The initial reaction is mediated by a haloalkane dehalogenase (DhlA); the final detoxifying reaction by haloacid dehalogenase (DhlB; see [33] and references therein). The introduction of *dhlA* into *Arabidopsis* resulted in the production of 2-chloroacetaldehyde that proved to be toxic to the plants [31]. Toxic effects were observed in tobacco (*Nicotiana tabacum* cv. *Xanthii*) expressing only *dhlA*, but not dual transformants expressing both *dhlA* and *dhlB* [32••].

This group of studies resulted in several significant findings. First, genetic modification can impart metabolic activity against VOCs that might otherwise be recalcitrant in higher plants. In the studies with CYP2E1 and the DhlA/DhlB systems there was little evidence of uptake or degradation of contaminants by untransformed plants, though the same contaminants were readily degraded by the transformed plants. Apparent product toxicity was observed with the DhlA-only tobacco, and CYP2E1 poplar exposed to VC, suggesting possible deleterious effects of transformations. In each case the exposure level was relatively high compared to what might be expected at contaminated sites, so it is unclear whether it would be a concern in field applications.

Second, the transgenic enzyme systems appear to interact with native enzymes, thereby maintaining metabolic activity. The CYP2E1 transformations included only CYP2E1 and not the genes for mammalian NADPH-P450 reductase or cytochrome b₅, which are essential in the CYP2E1 electron transport chain. Nonetheless, the modified plants were capable of degrading a wide variety of contaminants. Apparently the NADPH-P450 reductase and cytochrome b₅ expressed in tobacco and poplar are sufficiently homologous to maintain near-complete functionality of mammalian CYP2E1 in plants. The lack of activity toward TCA, a known metabolite of CYP2E1, may indicate some functional modification.

A third finding is that phase I activity may be rate-limiting in the metabolism of xenobiotics in plants. The increase in CYP2E1-mediated oxidation resulted in an overall increase in the rate of uptake or loss. Trichloroethylene exposure led to the increase of chloral and trichloroethanol, the oxidative metabolites, but also bound trichloroethanol-glucoside suggesting that the conjugation and transport/compartimentalization reactions have higher maximum rates than demonstrated in controls. Therefore, increased metabolism of VOCs may be brought about by an increase in phase I activity. The genetic modification of plants with the specific aim of increasing phase II or phase III activity for the remediation of small organics is rare, though it has been investigated for the enhanced phytoremediation of agricultural chemicals or inorganic contaminants (see other reviews in this issue).

Finally, several studies have indicated that unmodified poplars can degrade VOCs in water that is taken up in the transpiration stream [34,35], an uptake that may involve aquaporins. It is unclear whether increased *in planta* metabolism due to genetic modification will result in increased uptake of organic contaminants in field applications, or whether transport may limit the overall rates.

Enhanced *ex planta* metabolism

Ex planta phytoremediation techniques includes plants genetically modified to overexpress genes for extracellular enzymes such as laccases and peroxidases from plants, or fungal or microbial species. This strategy may overcome mass transfer limitations as contaminants do not have to be taken up in the roots to be affected by enzymatic activity. The activity of laccase or peroxidases is generally non-specific, which could limit the degradation of specific contaminants in the rhizosphere. Although work has focused on the degradation of phenolic compounds, aliphatic hydrocarbons may be degraded by peroxidases from white rot fungi [36–38] though the mechanism of PCE or TCE degradation is debated [39].

A transgenic *Arabidopsis* was developed which expressed a secretory laccase, *LAC1*, from cotton (*Gossypium arboreum*) [40••]. Culture media from LAC1 plants showed laccase activity whereas there was no measurable activity in media from wild types, suggesting that laccase was secreted. The LAC1 plants showed enhanced resistance to a variety of phenolics compared to wild types in growth media. Wild-type and *LAC1*-expressing plants were grown in soil and sprayed with trichlorophenol, which is phytotoxic. The wild-type plants showed severe chlorosis while the damage to the LAC1 plants was less severe. The data suggested that LAC1 secreted from the transgenic plants metabolized the trichlorophenol *ex situ* resulting in a less toxic environment for plant growth. In a similar study, the lac-case of *Coriolus versicolor* was expressed in tobacco resulting in the secretion of laccase into the rhizosphere and the enhanced degradation of bisphenol A and pentachlorophenol (PCP) in hydroponics [41]. Degradation in soils was not examined. Hirai *et al.* reported the more-efficient expression of a model laccase (*scL*) from *Schizophyllum commune* in tobacco by utilizing a mutagenized *scL* (*scL12*) sequence with a decreased CpG-dinucleotide content by 12% [42•]. Apparently gene sequences high in CpG content are not expressed efficiently in plants. The transformants with the *scL12* sequence degraded trichlorophenol to a higher degree than the *scL* or wild-type plants.

Oller *et al.* developed a transgenic tomato (*Lycopersicon esculentum* Mill. cv. Pera) that overexpressed *tpx1*, a native peroxidase, resulting in higher peroxidase activity [43]. Hairly root cultures from one clone removed phenol from hydroponics at a higher efficiency than wild type hairy root cultures.

A manganese peroxidase gene (*MnP*) from *Coriolus versicolor* was expressed in tobacco plants [44]. The MnP activity in media containing transgenic root cuttings was over 50 times greater compared to controls suggesting the secretion of the MnP enzyme. A similar incubation in the presence of PCP resulted in approximately 2-fold reduction in PCP concentration with the transgenic line compared to controls. The same laboratory group transformed poplar (*Populus seiboldii* × *Populus gradientata*) with *MnP* from *Trametes versicolor* [45••]. Several transgenic lines had higher MnP activity than the control and contributed to a more-rapid removal of bisphenol A from hydroponic media.

This second suite of studies suggests that the enhanced secretion of *ex situ* laccase and peroxidase enzymes may be a valuable tool in phytoremediation of small organic pollutants, though pollutant degradation rate increases were modest (less than fivefold). Collectively they have demonstrated that extracellular enzymatic activity is increased through genetic manipulation and that this increase can lead to the degradation of important pollutants and the increased ability for plants to grow in otherwise phytotoxic environments. The effects on excreted proteins or cofactors of inhibitory substances, or contaminant sorption and availability, in complex soil environments, has yet to be determined.

Considerations

The efficacy of either increased *in planta* metabolic activity or *ex planta* enzymatic secretions may be maximized against soil and groundwater pollution by the root-specific expression of transgenes. Phytoremediation-related plant transformations have largely utilized the CaMV 35S promoter to drive constitutive expression in most plant tissues. There is evidence transgene expression under CaMV 35S promoter in root tissue may be less than in leaves [46,47]. Other promoters such as ubiquitin 3 (UBQ3) from *Arabidopsis* [48] or the *rolD* of *Agrobacterium rhizogenes* [49] are active in roots and may be valuable for obtaining high, root-specific activities, though functionality remains to be investigated in trees.

Finally, the optimization of phytoremediation using genetic modifications may require the introduction of several genes for transport, multistep metabolic pathways, and sequestration.

Transgene stacking, in which multiple traits are conferred to plants by the expression of two or more foreign genes, has been used to develop plants for agricultural applications, though problems, such as silencing, have been encountered [50].

Conclusions

Several experiments have demonstrated the ability of transgenic plants and trees to degrade environmental contaminants that are either recalcitrant, or poorly degraded by native plant enzymatic systems. Work has focused on enhancing *in planta* metabolic activity by the expression of transgenes known to be involved in phase I transformations, or *ex planta* activity by increasing the secretion of native or microbial enzymes. Mass-transfer limitations on plant uptake of pollutants reduce the effectiveness of activity *in planta*. Mechanisms by which pollutants enter plant roots are poorly understood. Additional genetic modification may provide increases in plant uptake and degradation. Increases in pollutant degradation activity *ex planta* by transgenic plants have been modest and for a handful of the wide variety of pollutants found to be susceptible to laccase and peroxidases activity in fungal cultures. It would be valuable to increase the scope of substrates investigated to include a wider variety of contaminants and also to ascertain whether increased pollutant degradation can be maintained in soil systems. Field studies are needed to more-clearly ascertain whether either strategy will translate to enhanced phytoremediation in the field.

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Figure 1.

Genetic modifications to enhance phytoremediation of small organic contaminants. Demonstrated *in planta* activity includes the expression of mammalian cytochrome P450 2E1 (CYP2E1) gene resulting in the enhanced metabolism of trichloroethylene (TCE), vinyl chloride (VC), and benzene [26,28,30•]. Expression of a bacterial gene for haloalkane dehalogenase (DhIA) and haloacid dehalogenase (DhIB) resulted in the complete dehalogenation of 1,2-dichloroethane (1,2-DCA) that was otherwise recalcitrant [32••]. Demonstrated *ex planta* activity was brought about by the expression of laccase (LAC), peroxidase (PER), or manganese peroxidase (MnP) encoding transgenes in plants and secretion into the rhizosphere. Laboratory evidence suggested a more-rapid degradation of bisphenol A (bisA), and trichlorophenol and pentachlorophenol (TCP, PCP) [40••,41,42•, 43,44,45••,47].