Degradation of Low Molecular Weight Volatile Organic Compounds by Plants Genetically Modified with Mammalian Cytochrome P450 2E1

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Cytchrome P450 2E1 (CYP2E1) is a key enzyme in the mammalian metabolism of several low molecular weight volatile organic compounds (VOCs), such as trichloroethylene (TCE), vinyl chloride (VC), carbon tetrachloride (CT), benzene, chloroform, and bromodichloromethane (BDCM), which are all common environmental pollutants that pose risks to human health. We have developed a transgenic tobacco (Nicotiana tabacum cv. Xanthii) that expresses CYP2E1 with increased activity toward TCE and ethylene dibromide. In experiments with tobacco plant cuttings exposed to VOCs in small hydroponic vessels, the transgenic tobacco had greatly increased rates of removal of TCE, VC, CT, benzene, toluene, chloroform, and BDCM, compared to wild-type or vector control tobacco, but not of perchloroethylene or 1,1,1-trichloroethane.

Introduction

Low molecular weight volatile organic compounds (VOCs) such as trichloroethylene (TCE), carbon tetrachloride (CT), benzene, and chloroform are common pollutants in the air, soil, and groundwater. Exposure can lead to adverse health effects; all are listed either as known or possible human carcinogens. Trichloroethylene is a widespread organic pollutant, having been used for years as a cleaning solvent. It is commonly found at United States Environmental Protection Agency Superfund sites and is one of the pollutants most frequently detected in groundwater (1, 2). Carbon tetrachloride was widely used as a dry cleaning solvent and precursor for the production of chlorofluorocarbons during the last century. Although use and production have been phased out through a variety of regulations, CT is still a common groundwater pollutant (3). Benzene is associated with petroleum contamination and is found in soil and groundwater at numerous leaking underground storage tank sites. Benzene is a leading gaseous pollutant of indoor air due to fuel storage in attached garages and environmental tobacco smoke (4). In addition to being a frequent contaminant of groundwater, chloroform is a disinfection byproduct formed during the chlorination of drinking water and wastewater. Chloroform can be volatilized into indoor air during household water use, especially in showers or other hot water applications. Fiss et al. (5) found that use of household products containing triclosan, a common antibacterial agent, could result in the production of sufficient chloroform to present a significant exposure risk through the air.

There are many traditional treatment technologies, such as pump-and-treat and excavation, available to address sites contaminated with VOCs, but they may be cumbersome, expensive, and time-consuming to apply. Phytoremediation is an innovative treatment that uses plants and plant-related processes (e.g., rhizosphere activity, transpiration) to remediate contaminated sites; it is potentially less intrusive, cheaper, and more widely accepted. However, there are limiting factors such as extensive land requirements for planting and a limited growing season, which may restrict its employment. To help optimize phytoremediation applications, plants have been genetically modified to increase metabolism of specific contaminants (6, 7). Transgenic tobacco plants expressing the nitroreductase *nsfI from Enterobacter cloacae showed a marked improvement in the ability to tolerate and transform TNT (8). An Arabidopsis thaliana transformed to express the bacterial gene *xplA, which encodes for a cyclotrimethylenetrinitramine (RDX)-degrading cytochrome P450, has been shown to degrade RDX whereas vector control plants only sequester small amounts of RDX (9). Transgenic rice plants expressing human cytochrome P450 isoforms 1A1, 2B6, and 2C19 were found to improve the phytoremediation potential of the herbicides atrazine and metolachlor in soils (10). Doty et al. (11) have shown that tobacco transformed to express mammalian cytochrome P450 2E1 (CYP2E1) rapidly metabolizes TCE and ethylene dibromide. These plants are the subject of this study.

Cytochrome P450 2E1 is an enzyme that is significant in the degradation of xenobiotics in mammalian systems, including TCE, CT, 1,1,1-trichloroethane (TCA), chloroform, and benzene (12). The goal of this work was to evaluate the activity of transgenic tobacco against various organic pollutants. Sterile apical cuttings from either modified or unmodified tobacco plants were placed in sealed vials with liquid growth media and dosed with VOCs. The headspace concentration was measured over time and the normalized loss rates were calculated and compared.

Materials and Methods

Plant Material. Transgenic, vector control, and wild type tobacco plants (Nicotiana tabacum cv. Xanthii) were used in this experiment. Plant transformations were as described in Doty (11). Briefly, plasmid pSLD3 containing cDNA for human CYP2E1 under control of the Mac promoter was introduced into tobacco leaf discs via Agrobacterium mediated transformation. Transformations were verified by polymerase chain reaction and the plants regenerated. The CYP2E1 expression and TCE transformation capability of several lines were evaluated and the best-performing line was self-crossed and progeny, designated 3–1–2, were used for further study (11). Vector control plants were transformed with plasmid pCGN1578, which did not contain cDNA for CYP2E1. Wild type plants were not transformed.

Sterile plant material was obtained by propagating apical cuttings from whole plants in sterile vessels with amended growth media consisting of Murashige and Skoog (MS) + vitamins (Caissons MSP002), 30 g L−1 sucrose, 1 g L−1 phytoestrogen gellan gum, 3.5 g L−1 phytagar, 1 mL L−1 Fungigone (Bioworld, Dublin OH), and 1 mM indole-3-butyric acid. Apical cuttings
were placed in water in 50 mL plastic centrifuge tubes (Sarstedt, Newton, NC) for 24 h prior to sterilization. Sterilization was performed by submerging the cuttings for 10 min in 10% bleach solution (final concentration 0.35% NaClO), rinsing with sterile, deionized water, 5 min in 1% Iodophor solution, rinsing three times with sterile, deionized water, and transferring into a sterile growth vessel. All work was performed in a sterile, laminar-flow hood. Plants grew in 6–8 weeks and served as sterile source material for the vial experiments. Cuttings for the vial experiments were obtained from the apical ends of the sterile plants. Each apical cutting had 3–5 leaves and wet mass from 1.5 to 2 g.

**Experimental Setup.** Sterile plant cuttings were placed in clear glass volatile organic analysis (VOA) vials, 40 mL nominal volume, in 10 mL of half-strength MS basal salt mixture with 30 g L\(^{-1}\) sucrose (pH 5.7). Vials were capped with 24 mm MiniInert valves with silicone septa (VICI Valco Instruments, Houston, TX) and held for 24 h prior to chemical exposure. Cuttings were incubated at approximately 23 °C under fluorescent lights with a 16-h photoperiod.

Several chemicals were investigated in this study including perchloroethylene (PCE), TCE, vinyl chloride (VC), CT, TCA, chloroform, bromodichloromethane (BDCM), benzene, and toluene. For each experiment, a set of at least three transgenic and three vector control plant cuttings was set up, with one cutting per vial, and injected with known amounts of chemical and three vector control plant cuttings was set up, with one cutting per vial, and injected with known amounts of chemical.

**Sampling and Analysis.** Initial sampling occurred at least two hours following the introduction of chemicals into the VOA vials to allow air–liquid equilibrium. Subsequent sampling frequency was either 24 or 48 h, according to the uptake rate of the chemical of interest. Samples were obtained by withdrawing a known volume of vial headspace with a gastight glass syringe and injecting manually into a gas chromatograph (GC) for analysis. Headspace samples from vials containing PCE, TCE, CT, TCA, and chloroform were analyzed with a GC–electron capture detector (ECD). Two GC–ECDs were used during the course of the experiments: Perkin-Elmer AutoSystem XL GC–ECD with a Supelco PTE-5 column (30 m, 0.32 mm i.d., 0.32 μm film thickness); and, SRI 8610C with a Supelco SPB-624 column (60 m, 0.53 mm i.d., 3.0 μm film thickness). Headspace of vials containing VC, benzene, and toluene was analyzed with a SRI 8610C GC–flame ionization detector (FID) with a Supelco Alumina Sulfate Plot column (50 m, 0.53 mm i.d.). The GC oven temperatures were isothermal for all experiments. Sample injections were performed in triplicate.

**Analysis.** Analysis for chloroethene (TCOH) was performed in the TCA degradation experiments. Plants were removed from vials, flash frozen in liquid nitrogen, and ground in a mortar and pestle. Approximately 1.5 g of frozen tissue was placed in a chilled centrifuge tube with 2 mL of 1 N H\(_2\)SO\(_4\)/10% NaCl and shaken vigorously for 1 min. Methyl tert-butyl ether (MTBE; 10 mL) was added and the mixture was shaken for 1 min, followed by centrifugation at 8000 rpm for 10 min. The supernatant (7 mL) was transferred to a septa-capped, amber glass vial containing 2 g of Na\(_2\)SO\(_4\) and stored for 1 h. An aliquot was transferred to an autosampler vial for analysis on a GC–ECD. The GC oven temperature program was 40 °C for 1 min, ramped at 10 °C minute\(^{-1}\) for 19 min, and held at 230 °C for 4 min. Analysis for BDCM was performed by solvent extraction due to difficulties in optimizing the peak response with manual injection in the SRI GC–ECD. Initially, cuttings were placed in 15 mL of liquid media. Following chemical dosing and equilibration, 5 mL of media was extracted through the septa with a gastight syringe and mixed with 5 mL of 1 N H\(_2\)SO\(_4\)/10% NaCl in a 40 mL amber glass vial and shaken vigorously for 1 min. Ten mL of MTBE was added, the mixture was shaken for 1 min, and allowed to stand for 10 min. Seven mL of MTBE was transferred, held with Na\(_2\)SO\(_4\), and analyzed by GC–ECD as described above for TCOH.

**Analysis of the Uptake Kinetics.** Analysis of the uptake kinetics was performed by a regression analysis of the natural-log transformed concentration data over time. The significance of the regression was verified through analysis of variance testing for each regression at a significance level of 0.05. First-order chemical loss

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**TABLE 1. Initial Mass Dosing (µg) and Equilibrium Liquid Concentration (µg/mL) of the Compounds Tested in This Study**

<table>
<thead>
<tr>
<th>compound</th>
<th>initial mass (µg)</th>
<th>equilibrium liquid concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>perchloroethylene</td>
<td>54</td>
<td>1.64</td>
</tr>
<tr>
<td>trichloroethylene</td>
<td>125</td>
<td>5.6</td>
</tr>
<tr>
<td>vinyl chloride</td>
<td>1000</td>
<td>22</td>
</tr>
<tr>
<td>chloroform</td>
<td>0.25</td>
<td>0.0025</td>
</tr>
<tr>
<td>carbon tetrachloride</td>
<td>10</td>
<td>0.2</td>
</tr>
<tr>
<td>bromodichloromethane</td>
<td>0.6</td>
<td>0.05</td>
</tr>
<tr>
<td>1,1,1 - trichloroethane</td>
<td>16</td>
<td>0.5</td>
</tr>
<tr>
<td>toluene</td>
<td>15</td>
<td>0.9</td>
</tr>
<tr>
<td>benzene</td>
<td>85</td>
<td>1</td>
</tr>
</tbody>
</table>

**FIGURE 1. Degradation of chloroethenes TCE (A) and VC (B) by CYP2E1-expressing and vector control or wild type tobacco plant cuttings over time. Chemical dosing occurred at time zero. Sample collection began 2–12 h after initial dosing to allow air–liquid equilibrium. Points are the average mass of individual vial tests (n = 4 TCE; n = 3 VC); error bars indicate ± 1 standard deviation.**
rates were normalized for plant mass and compared using Student’s t statistic at a significance level of 0.05.

**Results**

**Chloroethenes.** Tobacco plant cuttings genetically modified with CYP2E1 displayed significant degradation of TCE, while the vector control plant cuttings did not display activity significantly different from that of the no-plant controls (Figure 1A). Similarly, tobacco plant cuttings expressing CYP2E1 degraded VC, while the wild type did not demonstrate statistically significant degradation (Figure 1B). There was no removal of PCE by any of the plants tested (data not shown). Analysis of the log-transformed data indicated that transformation kinetics were first-order for TCE \( r^2 = 0.93, P < 0.05 \) and VC \( r^2 = 0.99, P < 0.05 \).

**Haloalkanes.** Chloroform (Figure 2A) and BDCM (Figure 2B) were degraded in vials containing the CYP2E1-transformed plant cuttings, while there was no significant removal in vials with either the wild type or vector control plant cuttings. The rate of removal of CT (Figure 2C) by plant cuttings expressing CYP2E1 was significantly greater than that of either the no-plant or vector controls assuming first-order kinetics \( P < 0.05 \). The normalized degradation rate of CT was 3.3 times greater for the plant cuttings expressing CYP2E1 compared to the vector control plant cuttings. Analysis of the log-transformed data indicated that chloroform \( r^2 = 0.88, P < 0.05 \) and CT \( r^2 = 0.98, P < 0.05 \) degradation followed first-order kinetics. The experimental method used to measure BDCM precluded analysis of uptake kinetics.

**Aromatic Hydrocarbons.** Benzene and toluene were almost completely removed within 4 days from vials containing CYP2E1-transformed plants (Figure 3A and B). The control plant cuttings did not show any activity toward benzene. Toluene was degraded by the vector control plant cuttings, though at a significantly lower rate than the CYP2E1-transformed plant cuttings \( P < 0.05 \). The first-order removal rate was 10.4 times higher for the CYP2E1-transformed plant cuttings compared to the vector control plant cuttings. Analysis of the log-transformed data indicated that benzene \( r^2 = 0.97, P < 0.05 \) and toluene \( r^2 = 0.98, P < 0.05 \) degradation followed first-order kinetics.

**Discussion**

These results demonstrate the increased degradation of a wide variety of VOCs by genetically modified plants that express mammalian CYP2E1. For most of the chemicals investigated, the transgenic plant cuttings displayed either increased activity, or were able to degrade pollutants that were otherwise recalcitrant to the wild type or vector control tobacco plant cuttings. The greater activity conferred by CYP2E1 expression has the potential to improve the effectiveness of phytoremediation in the cleanup of soil, groundwater, and indoor air.

The enhanced metabolism of TCE by plants expressing CYP2E1 has been previously reported (11). The replication
While other P450s can be stimulated by either holo-b5 or other P450s, CYP2E1 may only be enhanced by holo-b5, apo-b5, or even through protein–protein interactions with b5. Several of the P450 enzyme isoforms, including 2E1, require b5 to carry out their full range of activities, though xenobiotics, likely utilizing native plant homologues to NPR and b5, to facilitate electron transport. However, differences between the homologous versions found in plants compared to those in humans possibly causes conformational changes that inhibit catalytic activity toward TCA.

Benzene and toluene can be taken up by plants and released to the atmosphere with limited transformation (16). Toluene volatilization through leaves can be reduced by inoculating plants with endophytes expressing the toluene-degrading pTOL plasmid, thereby increasing in planta degradation (17). Similarly, the improved degradation of benzene and toluene by CYP2E1-transformed plants shown here could also reduce transpiration and increase phytoremediation effectiveness.

The trihalomethanes (THMs) chloroform and BDCM were degraded by the transgenic plant cuttings. Trihalomethanes, which may be present as disinfection byproduct in chlorinated drinking water, can volatilize into indoor air. In preliminary work, we found a 9-fold increase in average daily chloroform concentration in the air with shower use (data not shown; BDCM was not measured). Studies have looked at the potential of wild type household plants for the remediation of contaminants such as formaldehyde, benzene, and TCE, from indoor air (18, 19). Assuming that plant degradation was limited by the endogenous enzymatic activity, transgenic plants similar to those described in this paper may have application in the remediation of indoor air.

Overall, our findings suggest potential applications for genetically modified plants in the field. The CYP2E1-transformed tobacco plants have demonstrated activity against broad classes of environmental pollutants such as chlorinated solvents (both alkenes and alkanes) and aromatic hydrocarbons suggesting that CYP2E1-transformed plants could be used to remove pollutants faster in environments contaminated by single or mixed contaminants, particularly when cleanup is limited by planting area or time. It is important, however, to acknowledge the limitations of this study, which was designed specifically to investigate differences in plant metabolism. Field applications are complex; plant metabolism alone may not be the determining measure of success.

The spread of transgenic material may be a concern, particularly with plants with an annual reproductive cycle, such as the tobacco. Transgenic trees that express CYP2E1 could combine the higher metabolic activity with deeper root systems, while having a more controlled reproductive cycle. This is potentially quite desirable. This study and others, which have reported on various applications of genetically modified plants for detoxification of polluted environments (9, 10), demonstrate the potential of transgenic modifications to increase the range and utility of phytoremediation.

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Literature Cited


