Transgenic phytoremediation blasts onto the scene

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The EPA National Priority List contains 22 ammunition production and processing sites that are laden with explosive and propellant wastes. With levels of 2,4,6-trinitrotoluene (TNT) contamination as high as 200 g/kg of solids, some of these sites are literally on the verge of exploding. They also present serious exposure risks to humans and wildlife, as many of these contaminants are also strong toxins and mutagens. In this issue, French et al. describe a new option for cleaning up this dangerous mixture: the use of transgenic plants. They engineered plants to express a bacterial enzyme that can completely denitrify TNT and trinitroglycerin (GTN) into harmless compounds.

Bioremediation of explosives is not a new idea. Until recently, workers have focused on the ability of fungi and microbes to destroy high-energy compounds such as TNT, hexahydro-1,3,5-triazine (RDX), N-methyl-N,2,4,6-tetranitroaniline (TETRYL), and octahydro-1,3,5,7-tetranitro-1,3,5,7 tetrazocine (HMX). Unfortunately, current technologies require the excavation of the contaminated soil and processing in engineered bioreactors. Not surprisingly, these procedures tended to be just as expensive as other more traditional incineration methods. Conceptually at least, phytoremediation is well suited to munitions contamination, since much of the latter is spread over large areas in near-surface soils. These include areas where off-specification materials were detonated, soils surrounding munitions manufacturing and packing facilities, and locations associated with abandoned disposal ponds and drainage ditches[1]. Treatment of this type of contamination by the traditional method of excavation and incineration is expensive and results in excessive exposure of site workers to toxins.

To reduce these risks, several investigators have explored the use of wild-type plants to destroy munitions wastes. Goel et al.[2] reported that cultured sugar beet cells could degrade GTN, and Hughes et al.[3] found that aquatic plants and hairy root cultures can similarly transform TNT. But these successes were limited by the fact that GTN could only be denitrated to di- and mononitrated glycerol; complete removal of nitrate groups was never achieved[2]. Even worse, the TNT degradation actually led to accumulation of another toxin—aminonitrotoluene[3]. A further limitation was the fact that plants naturally take up and metabolize nitrated organics at a snail's pace—on the order of 10 times slower than microbes.[2]

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Figure 1. The majority of plant-based cleanup has been applied to hyperaccumulation of metals in specific plant portions, which represents displacement rather than destruction of contaminant species and still requires disposal under stringent guidelines.

Bioremediation of heavy metals was similarly limited, until a recent report by Rugh et al.[4] described the use of genetic engineering for phytoremediation of mercury. Yellow poplar saplings expressing the bacterial mercuric reductase gene were generated and shown to take up mercury and transport it efficiently out of the soil. Unfortunately, the contaminant was not destroyed but merely transformed from soil-bound ionic mercury, Hg^{2+}, to airborne elemental mercury, Hg^0. For phytoremediation to be a viable, cost-effective alternative, plants will need to destroy the contaminants rather than simply accumulate or displace them.

The work of the Bruce team, as described in this issue, represents the first report of genetically modified plants for the transformation of xenobiotic contaminants to nontoxic materials. By expressing the bacterial pentaerythritol tetranitrate (PETN) reductase gene in tobacco, these researchers have created strains that are more resistant to high concentrations of explosives such as GTN and TNT and that have the potential to remove nitrate completely and degrade these toxic compounds. The beauty of expressing PETN reductase in tobacco is that it encodes the only known denitration enzyme for TNT. In contrast, other known TNT-degrading enzymes such as nitroreductases typically result in partial reduction of nitro groups, yielding various aminated and nitrated toluene compounds that are both toxic and recalcitrant to further degradation.

Clearly, the work by French et al.[5] is a step in the right direction, and plants of this type will find application at locations such as TNT manufacturing facilities where TNT and less nitrated toluenes are the only soil contaminants. However, there are numerous other locations where TNT is only one component in a mixture of explosive contaminants. Other recalcitrant compounds that are often included in such mixtures are RDX, TETRYL, and HMX. For phytoremediation to provide effective cleanup at these sites, plants must mediate uptake and metabolism of multiple compounds. All of these chemicals are transformed by biological systems. However, biochemical contaminant destruction mechanisms have not been fully identified. Developing strains of plants that are capable of degrading a wide range of explosives including highly recalcitrant RDX, TETRYL, and HMX will require the identification, characterization, and successful cloning of the encoding regions of several reductases.

Introducing metabolic pathways into plants for phytoremediation of highly recalcitrant compounds such as RDX, TETRYL, and HMX is certainly possible considering the current state of plant molecular biology research. Chen et al.[6] have demonstrated the introduction of up to 13 different transgenes in rice by using particle bombardment techniques. Although these genes functioned independently, multiple genes requiring synergistic function have been introduced into plants for a variety of purposes. These include complex protein production such as secretory immunoglobulin, which recognizes the native streptococcal antigen I/II cell surface adhesion molecule[7], pathway introduction such as the polyhydroxybutyrate production pathway from *Alcaligenes eutrophus*[8], and trait modification such as the introduction of C_4 photosynthesis pathways into C_3 plants (Ku et al., personal communication). Given the existing advances in plant molecular biology, the use of transgenic phytoremediation for highly recalcitrant munitions could soon become a reality.

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