Improving phytoremediation through biotechnology

Editorial overview

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Phytoremediation of contaminated soils offers an environmentally friendly, cost effective, and carbon neutral approach for the clean up of toxic pollutants in the environment. Plants with abilities to hyperaccumulate heavy metals, uptake volatile organic compounds, and sequester pollutants have been proposed as a solution to the treatment of toxic contamination in situ. However, the use of plant-based technologies has a number of limitations, primarily due to the fact that plants are autotrophs and not ideally suited for the metabolism and breakdown of organic compounds. One of the major limitations with current phytoremediation is the often slow time-scale for remediation to acceptable levels and also toxicity to the plants themselves. To some extent, this can be addressed through interactions with the natural micro flora associated with plants; both endophytic bacteria and rhizosphere bacteria have been shown to have the potential to degrade organic compounds in association with plants.

The obvious approach to address these limitations is the application of recombinant DNA technology to express specific genes from heterotrophic organisms such as bacteria and mammals to increase plant tolerance and metabolism of organic chemicals or heavy metals. Herbicide tolerance is one of the most successful modifications to date. Much progress has been made as is highlighted in the reviews to follow, however, there is an urgent need for more field trials to evaluate the exciting developments coming out of the laboratory.

Phytoremediation of metals and metalloids

Selenium (Se) is a micronutrient found naturally in soils that is toxic at high levels. Se is chemically similar to sulfur, and it is thought that its toxicity is due to the misplacement of Se instead of sulfur in proteins. The strategies used for remediation of toxic levels of Se utilize information gleaned from natural Se hyperaccumulators. As described in the review by Pilon-Smits and LeDuc, methylation of SeCys and SeMet, preventing the misuse of these amino acid derivatives in proteins, conversion to volatile forms to remove the Se from soils, and the breakdown of Se compounds into innocuous elemental Se are all strategies employed through the expression of key enzymes in transgenic plants. Enhanced extraction of Se was achieved not only at the lab-scale but also in field trials. The increased Se accumulation had no negative impact on overall biomass accumulation. The ecological impact of the transgenic plants was also studied. Although some Se-tolerant specialist insects were identified, overall, the Se-accumulating plants deterred herbivory from a wide range of animals.

Mercury is a highly toxic pollutant with expensive clean up. The toxicity varies depending on the form, from the least toxic elemental form that is less...
bioavailable, to the highly toxic organomercurial compounds that can concentrate as it moves up the food chain. The strategies employed for dealing with this form of pollution are reviewed by Ruiz and Daniell and rely on the ability of some bacteria to convert the mercury to different forms. By expressing these bacterial genes, namely the merA gene encoding mercuric ion reductase and the merB gene encoding organomercurial lyase, transgenic plants had improved resistance to the toxic effects of mercury. Resistance was further improved when merB was expressed in the endoplasmic reticulum. This result as well as the knowledge that the chloroplast is the primary target for mercury poisoning helped lead the research in the direction of plastid genome engineering. Greatly improved resistance to mercury was achieved with high expression levels of merA and merB in the chloroplast. Engineering of the plastid genome has many advantages over the traditional engineering of the nuclear genome. Bacterial genes, such as merA and merB, are expressed more efficiently in plastid genomes that are believed to be of bacterial origin. Very high expression levels can be achieved through plastid engineering without the issue of gene silencing. Furthermore, since the plastid genomes are maternally inherited, there is no transgenic escape via pollen. The efficiency of resistance is greatly reduced compared to nuclear transformation. But with the continued development of this method, transformation efficiency is improving. By expressing merA and merB in the plastid genome, transgenic tobacco plants exhibited much higher resistance to phenyl mercuric acetate and were able to efficiently transport organic mercury and then volatilize elemental mercury. Continued improvements are being made to improve the effectiveness of phytoremediation of mercury using transgenic technologies.

Arsenic in the environment is a major environmental pollutant affecting millions of people globally. Phytoremediation may offer a practical cost-effective solution to clean up contaminated soils. The review by Zhu and Rosen focuses on recent progress developing transgenic plants for improved compartmentation and translocation of arsenic. Various strategies are presented including sequestration in the vacuole by complexation of arsenic through conjugation with glutathione (GSH) or phytochelatins and arsenate reduction using bacterial genes such as ArsC from Escherichia coli with co-expression of γ glutamylcysteine synthetase to provide sufficient GSH for subsequent conjugation. Translocation to the leaves is another approach by using an efflux homolog of ArsB and subsequent volatilization by methylation of arsenic to the gas trimethylarsine by introduction of the ArsM gene. These approaches in the laboratory lead to increased tolerance and removal of arsenic with the potential to reduce human exposure to arsenic through contaminated food and water.

Phytoremediation of herbicides, explosives, and small organic compounds

Herbicides play an important role in agriculture worldwide, but have negative effects as polluting agents on the environment. Herbicide tolerant transgenic plants have been developed and used in agriculture for many years and can enhance absorption and detoxification of herbicides leading to phytoremediation of polluted environments. The review by Hiroiuki discusses the genes and enzymes that have been used to engineer plants for herbicide phytoremediation. These include the introduction of mammalian cytochrome p450 s into rice and the improvement of Glutathione S-Transferase (GST) promoted conjugation by improving the synthesis of GSH by introduction of a γ glutathione synthetase into Poplar. Both of these approaches have led to the improved removal of atrazine and alachlor respectively. Other approaches present interesting potential including the expression of bacterial genes such as atrazine chlorohydrolase (atzZ) and genes that affect root development by increasing root mass through expression of bacterial 1-aminocyclopropane-1-carboxylate deaminase.

Explosives are toxic and persistent polluting contaminants in military sites worldwide where they can contaminate soil and groundwater. The review by Van Aken focuses on the development of transgenic plants for phytoremediation of 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine and glyceroltrinitrate by introducing and expressing bacterial nitroreductases and cytochrome p450 s. Plants expressing these genes show significantly increased tolerance, uptake, and detoxification of the target explosive. While most of the examples relate to model plants such as Arabidopsis the recent introduction of the pnrA gene encoding a nitroreductase from Pseudomonas putida into the fast growing tree Aspen has yielded promising results for practical applications in the field.

Small organic contaminants and volatile organic compounds such as chlorinated solvents like trichloroethylene (TCE) and carbon tetrachloride are widely used industrially and can be major contaminants of soil and groundwater. The review by James and Strand explores the approaches and strategies to generate transgenic plants to improve the phytoremediation of these pollutants. Similar to the removal of herbicides, the approach involves the expression of cytochrome p450 s such as human CYP2E1 in tobacco and poplar where transgenic plants show an increase in not only TCE metabolism but also metabolized vinyl chloride, benzene, toluene, and chloroform. The use of bacterial genes such as dhlAB for the degradation of 1,2-dichloethane from Xanthobacter conferred improved removal of this compound in plants. The authors also review the expression of extracellular enzymes, for example, laccases and peroxidases as an additional approach for phytoremediation of small organic compounds.
Plant associated microbes; rhizospheric and endophytic bacteria

Bioremediation of polychlorinated biphenyls (PCBs) in soils present a major difficulty due to the chemical nature of these pollutants. The use of plant–microbe strategies including rhizoremediation have been proposed by a number of laboratories. The review by Sylvestre and co-workers investigated the potential to optimize the process using transgenic plants and improved bacterial enzymes that would 1) initiate the PCB degradation process by expressing the genes for the first multi-component enzyme in the pathway (biphenyl 2,3-dioxygenase) releasing intermediates for further transformation by rhizosphere bacteria and 2) construction of transgenic plants harboring \( bphC \), a 2,3-dihydroxybiphenyl dioxygenase to overcome the inability of plants to cleave toxic dihydroxybiphenyls. The latter transgenic plants are more resistant to PCBs than wild-type plants indicative of the potential of these plants for improving the rhizoremediation of PCBs.

Endophyte-assisted phytoremediation is a promising new field to improve remediation by utilizing microorganisms that live within plants to improve plant growth, increase stress tolerance, and degrade pollutants. The review by Vangronsveld and co-workers describes some of the problems with phytoremediation and how they may be overcome using specific endophytes. Some challenges with traditional phytoremediation include the phytotoxic effects of certain pollutants, and the uptake but not degradation of pollutants within plants, leading to unacceptable levels of volatilized pollutants or concentration of the pollutant in tissues subject to herbivory. By adding endophytic bacteria known to degrade the target pollutant, increased tolerance to certain pollutants and decreased volatilization of harmful pollutants has been achieved. The approach was successfully used to increase plant tolerance to toluene by inoculating first yellow lupin plants and then poplar plants with an endophyte capable of degrading toluene, resulting in reduced evapotranspiration of the pollutant by these plant species. Inoculation of pea plants with an endophyte able to degrade the herbicide, 2,4-D, imparted on the plants an increased ability to remove the chemical from soil without signs of phytotoxicity. Endophytes could be used not only to remediate organic pollutants but may also be used to improve phytoextraction of metals.

Future directions

Work to date has largely focused on introduction of single genes/traits; however, to make real progress, a systems-wide approach using complete pathways for metabolism, targeted co-ordinate expression in specific compartments, for example, root, uptake, translocation, and sequestration need to be developed. It will be also important to be cognizant of the positive interaction of the micro flora associated with plants and how its biodegradation capacity can be enhanced by plant exudates both within the plant and in the rhizosphere. Another exciting development is that the outcome of genomic sequencing projects and metagenomics can identify and isolate novel gene and enzyme activities for improving phytoremediation.

The reviews presented here can give but a flavor of this rapidly developing field and we would like to acknowledge the contributions and inputs to this section from the authors concerned.