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REVIEW

Plant Hairy Roots for Remediation of Aqueous Pollutants

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Abstract Significant progress has been made in recent years in enhancing the ability of plants to tolerate, remove, and degrade pollutants. Plant root remediation of contaminated soils and groundwater shows great potential for future development due to its environmental compatibility and cost-effectiveness. Hairy roots are disease manifestations developed by plants that are wounded and infected by *Agrobacterium rhizogenes*. The application of transgenic hairy roots in phytoremediation has been suggested mainly because of their biochemical resemblance to the roots of the plant from which they are derived. The application of genetic engineering has greatly augmented removal rates of hazardous pollutants. In addition, the rhizospheric bacteria that live on or around plant hairy roots also lead to improved tolerance to normally phytotoxic chemicals and increased removal of pollutants. This paper provides a broad overview of the evidence supporting the suitability and prospects of hairy roots in phytoremediation of organic pollutants and heavy metals.

Keywords Cadmium · Hairy roots · Hyperaccumulator · Phenol · Phytoremediation

Introduction

Rapid industrialization has resulted in the release of large amounts of potentially toxic compounds into the biosphere, which cause toxicity to all living organisms. Nowadays, preventing the release of toxins into the environment is of primary concern (Krämer and Chardonnens 2001). Recognition of the ecological and human or animal health hazards of pollutants has led to development of various technologies for remediation. Conventional methods, including incineration, irradiation, solvent extraction, microbial degradation, chemical oxidation and activated carbon adsorption, are currently applied to remove pollutants from wastewaters. However, these methods have certain disadvantages, such as low efficiency and high cost, or generation of products that are even more toxic than the original pollutants (Oller et al. 2005; Eapen and Souza 2005). Consequently, the development of efficient and reliable technologies for the clean-up of such contaminated industrial wastewaters before discharge into the environment is of great importance. Phytoremediation is a newly evolving field of science and technology that uses plants to clean-up polluted soil, water, and air using energy from sunlight (Meaghe 2000; Doty 2008). Its primary advantage is that it is approximately ten times less expensive, and is safer, than conventional strategies (Doty 2008). Phytoremediation has been used to treat a variety of pollutants. Pollutants can be remediated in plants through several natural physiological and biochemical processes (Meaghe 2000). The genes involved in the metabolism of chemical compounds can be isolated from various organisms, including bacteria, fungi, animals, and plants, and these genes are then introduced into candidate plants. With the help of genetic engineering, plants can be used to extract, sequester, and/or detoxify a wide variety of aqueous contaminants. Phytoremediation is viewed widely as the ecologically responsible alternative to the environmentally destructive physical remediation methods currently practiced (Meaghe 2000) Table 1.

Among different biological systems used in investigating remediation processes, hairy root cultures are considered a

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Table 1 Selected examples of plant hairy roots used to remediate pollutants

Plant hairy roots	Pollutant	Effect	References
	Organic pollutants		
Catharanthus roseus	2,4,6-trinitrotoluene	Metabolism	Hughs et al. 1997; Bhadra et al. 1999
Carrot	Phenols	Metabolism	Santos de Araujo et al. 2002
Tomato	Phenols	Remediation	Talano et al. 2003; Oller et al. 2005; Paisio et al. 2010
Nicotiana tabacum	Tetracycline, oxytetracycline	Phytodegradation	Gujarathi et al. 2005
	2,4-Dichlorophenol	Phytodegradation	Talano et al. 2010
	Phenols	Metabolism	Alderete et al. 2011; Ibáñez et al. 2011
Cichorium intybus Brassica juncea	1,1,1-trichloro-2,2-bis-(40-chlorophenyl)ethane (DTT)	Phytodegradation	Suresh et al. 2005
Brassica juncea	Phenols	Metabolism	Singh et al. 2006
	Methyl orange	decolorization	Ghodake et al. 2009; Telke et al. 2011
Brassica napus	Phenols	Metabolism	Coniglio et al. 2008; González et al. 2012
Tagetes patula	Reactive Red 198	Phytodegradation	Patil et al. 2009
	Metals		
Thlaspi caerulescens	Cd	Uptake	Nedelkoska and Doran 2000
			Boominathan and Doran 2003a
Alyssum bertolonii	Ni	Uptake	Boominathan and Doran 2003b
Chenopodium amaranticolor	Uranium	Uptake	Eapen et al. 2003
Brassica juncea	Uranium	Uptake	Eapen et al. 2003
Carrot hairy root colonized with AMF *Glomus intraradices*	Cd	Metabolism	Janoušková and Vosátka 2005

remarkably valuable research tool (Zhou et al. 2011; Kang et al. 2011). Hairy roots develop as the consequence of the interaction between *Agrobacterium rhizogenes*—a Gram negative soil bacterium—and the host plant (Fig. 1). This transformation process leads to the emergence of "hairy roots" at the site of infection of the plant (Zhou et al. 2011). Hairy root cultures are isolated model systems with specific advantages, such as genetic and biochemical stability, rapid growth in dilute hormone-free medium under defined aseptic conditions, low costs of implementation, and easy maintenance, independence from other plant organs and from the microbiota, and the possibility of development in industrial bioreactor models (Oller et al. 2005). Hairy root cultures have proved to be excellent model systems in which to study phytoremediation, including several strategies of action, such as phytoextraction, phytodestabilization, as well as rhizofiltration of organic and inorganic pollutants (Santos de Araujo et al. 2002). Furthermore, growth and maintenance of such cultures is a cheap and straightforward procedure that allows studies to be carried out without the interference of microbiota (Santos de Araujo et al. 2002). Thus, hairy roots can contribute to developing methods for phytoremediation and to our understanding of the mechanisms involved in the removal, containment or decomposition of a wide variety of pollutants, such as elemental and organic pollutants. In addition, the genes involved in the metabolism of chemical compounds can be isolated from various organisms, including bacteria, fungi, plants, and animals, and these genes are then introduced into candidate plants that produce hairy root. The overexpression of these genes is required to enhance these natural properties. This review begins by first examining recent advances in enhancing phytoremediation through transgenic plant hairy root research and through the use of symbiotic endophytic microorganisms within plant roots.

Mechanisms of Root Remediation

Pollutants can be remediated in plant roots through several natural biophysical and biochemical processes: adsorption, transport and translocation; hyperaccumulation; or transformation and mineralization (Meaghe 2000) Fig. 2. Root surfaces specifically developed for uptake of elemental nutrients from soil and solutions have very large surface areas. Roots are the main organ to have contact with environmental pollutants and are also the site where the first reactions against the pollutant take place, among these production of bioactive molecules, e.g., enzymes such as laccases and peroxidases (Talano et al. 2006). During the process of adsorption, root surfaces bind many elemental pollutants as well as nutrients. Sunflower roots concentrate uranium 30,000-fold from

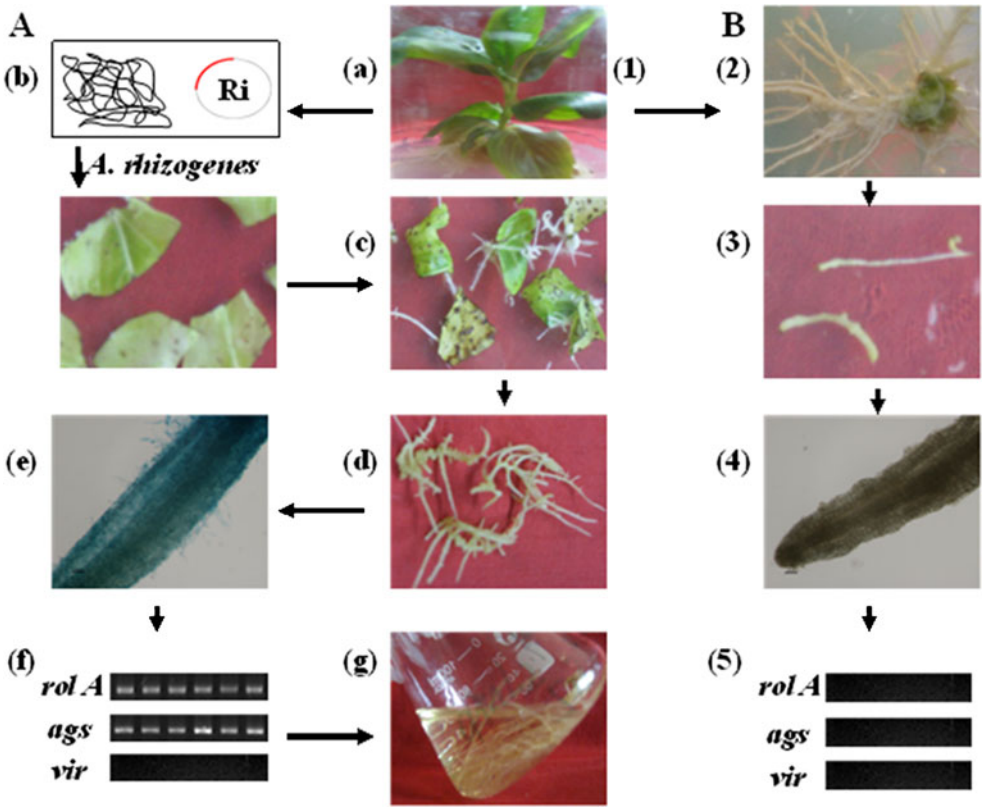

Fig. 1 Development of hairy root cultures (**a**) and plant roots (**b**). *a1* and (*b2* Aseptic cultivation of plants on hormone medium; *b* Pre-culture wounded plant tissues inoculated with *Agrobacterium rhizogenes*. *c* After 2–3 weeks, roots emerge at the wounding site and surrounding area. *d* Hairy roots (characterized by fast hormone-free growth, lack of geotropism, lateral branching and genetic stability) and plant root (*b3*) are excised to be cultured individually on selective and free hormone solid culture medium. *e* and *b4* Gus staining of putative positive lines. *f* and *b5* Genetic analysis to confirm the bacteria free hairy root clone. *g* Each positive clone is then transferred to liquid culture medium for obtaining established hairy roots

contaminated water (Dushenkof et al. 1997). Similarly, tobacco roots exposed to low concentrations of ionic mercury (Hg^{2+}) in liquid medium lowered the Hg^{2+} concentration of the medium nearly 100-fold in a matter of hours (Heaton et al. 1998). Indian mustard (*Brassica juncia*) can rapidly concentrate Cd^{2+}, Ni^{2+}, Pb^{2+}, and Sr^{2+} into root tissues at levels 500-times greater than those in the liquid medium (Meaghe 2000). Root surfaces are characterized by the presence of high affinity chemical receptors. Although research into the molecular physiology of root transport systems for elemental nutrients and pollutants is still in its infancy, excellent initial information is available on two related subfamilies of zinc transporter (ZIP) proteins that are involved in Zn^{2+} and Fe^{2+} uptake (Eng et al. 1998; Guerinot and Eide 1999). The metals are transported across the root cell membranes into the symplast. Roots of the zinc hyperaccumulator *Thlaspi caerulescens* contain more zinc transporters per gram fresh weight than the non-accumulator *Thlaspi arvense* (Lasat et al. 1996).

Plant roots secrete large quantities of citrate into their rhizosphere and again take up less Al^{3+}. The capacity of citrate to block Al^{3+} uptake has been confirmed independently in transgenic tobacco and papaya plants that overexpress a bacterial gene for citrate synthetase (De la Fuente et al. 1997). Citrate synthetase is a Kreb's cycle enzyme that combines an acetyl group and oxaloacetic acid to form citrate. The excess citrate in these transgenic plants is secreted into the growth medium and provides even greater resistance to $A1^{3+}$ toxicity (Meaghe 2000). In contrast to the behavior of citrate, most organic chelators increase metal ion uptake and translocation in plants. In response to nutrient metal ion deficiencies, plants root secrete phytosiderophores such as mugenic and avenic acids (Meaghe 2000). These metal-chelators increase the bioavailability of metals that are otherwise tightly bound to the soil and help to carry them into plant tissues. Synthetic chelators, such as ethylene diamine tetra-acetic acid

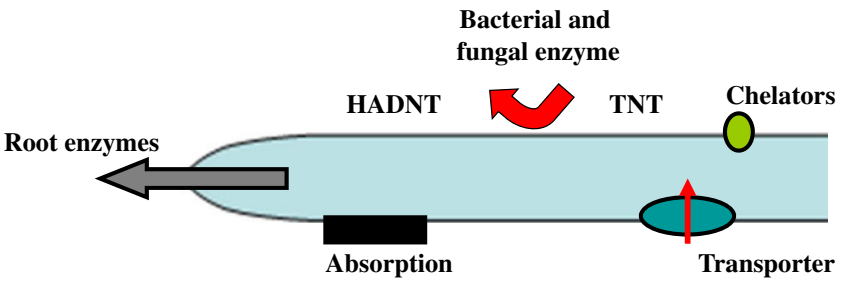

Fig. 2 Molecular mechanisms likely to be involved in remediation of pollutants by plant roots. *TNT* 2,4,6-trinitrotoluene; *HADNT* 2, 4-hydroxy dinitrotoluene

(EDTA), can mimic these effects. Thus, in contrast to the role of citric acid in reducing aluminum uptake, plants altered to increase their secretion of particular organic acids will probably demonstrate increased uptake and translocation of metal pollutants.

Another natural mechanism that offers exciting phytoremediation possibilities is the transformation in the rhizosphere of toxic elements into relatively harmless forms. Many elements (e.g., arsenic, mercury, iron, selenium, chromium) can exist in a variety of states, including different cationic and oxyanionic species and thio- and organometallics. These forms vary widely in their transport and accumulation in plants and in their toxicity to humans and other life forms (Meaghe 2000). The bacterial *merB* gene encodes an organomercurial lyase that degrades MeHg to methane and Hg^{2+}. This gene is expressed only in bacteria in conjunction with *merA* so that the final product of the bacterial mer operon is always Hg. Plant expression of *merA* and *merB* together produces tolerance of 50 times greater concentrations of MeHg than is required to kill controls plants, and 5 times greater than concentrations that kill *merB* plants (Bizily et al. 2000).

Organic pollutants can potentially be degraded chemically and ultimately mineralized into harmless biological compounds. The complex physiology and biochemistry of plant roots gives plants great potential as remediators of toxic organic pollutants. The best-characterized system involves a family of ATP-binding cassette (ABC) transporters often called the glutathione-S-conjugate pump. This system recognizes oxidized diglutathione (GS-SG), glutathione conjugates of organics, conjugates of diverse high-molecular-weight toxic organic xenobiotics, and peptide–metal complexes such as phytochelatins (Lu et al. 1998). The accumulation of toxic organics in plant vacuoles should favor their subsequent degradation. Plants can degrade nitroaromatic compounds (2,4,6-trinitrotoluene, TNT) that are highly toxic and carcinogenic, and notoriously difficult to metabolize. For example, hairy root cultures of *Catharanthus roseus* were capable of degrading most of the 25 ppM of TNT added to culture medium within a few days (Bhadra et al. 1999). Phytochelatins play a major role in metal detoxification in plants. It has been demonstrated that the phytochelatin synthase gene is induced by 20 μM $CdSO_4$, $CuSO_4$, and $ZnSO_4$ in *Pyrus betulaefolia* Bunge (Chang et al. 2012). Overexpression of a phytochelatin synthase gene in plant could be a good method to improve phytoremediation efficiency.

Hairy Root Remediation of Organic Pollutants

Organic pollutants can potentially be degraded and ultimately mineralized into harmless biological compounds. The complex physiology and biochemistry of plant roots gives plants great potential as remediators of toxic organic pollutants. Aromatic compounds, such as phenols, are included in the major classes of hazardous pollutants because of their carcinogenicity, recalcitrance to degradation and high toxicity. They are common synthetic contaminants in the effluents of a wide variety of industrial activities, e.g., coal conversion, petroleum refining, pulp and paper manufacturing, wood preservation, metal casting, resins and plastic production, among others (Wu et al. 1993; Miland et al. 1996).

Phenol and its chlorinated derivatives are used in the formulation of insecticides, herbicides and fungicides and can be found as toxic pollutants in industrial wastes and represent a potential danger to human health. Current methods for removal of phenols include solvent extraction, adsorption on activated carbon, chemical oxidation and also microbial degradation. Transformed carrot hairy roots were able to remove more than 90 % of the exogenous phenolic compounds from the culture medium within 120 h of treatment. Metabolism of these compounds occurred in the root tissue and was accompanied by an increase in peroxidase activity (Santos de Araujo et al. 2002). Plant peroxidases are localized mainly in cell walls and vacuoles. Peroxidases in the cell wall are very important in detoxification processes, because they are likely to be responsible for the formation of covalent links between hydroxylated pollutants and metabolites and plant cell wall polymers to form so-called 'inextricably bound residues' (Oller et al. 2005). One of the goals of phytoremediation with respect to peroxidases is to enhance the capacity of plant cells to lock pollutants into the bound residues. Hairy roots of tomato naturally contain high levels of peroxidases, and consequently are capable of removing phenols (Talano et al. 2003). After phenol treatment, peroxidase activity of tomato hairy root extracts decreased and isoenzyme patterns were affected. Neutral and basic peroxidases isoenzymes were slightly inactivated while the activities of acidic peroxidase isoenzymes remained unchanged. Removal assays and kinetic studies performed using root extracts and purified peroxidases suggest that basic peroxidase isoenzymes are the main peroxidases implicated in removal processes and that these could be inactivated during the treatment (González et al. 2006). However, Coniglio and his colleagues demonstrated that acidic peroxidase showed higher affinity and catalytic efficiency for phenol as substrate than neutral and basic peroxidases in *Brassica napus* hairy root cultures (Coniglio et al. 2008).

The overexpression of *tpx1* (tomato peroxidase 1, pI 9.6) correlated with higher in vivo and in vitro peroxidase activity in crude extracts of transgenic hairy root clones than in those of the wild type culture. Furthermore, peroxidase zymograms of transgenic clones showed higher activity of a band with pI 9.6 in the fraction ionically bound to cell wall proteins. Consequently, tomato hairy root cultures overexpressing *tpx1* increase phenol removal from aqueous solutions

(Oller et al. 2005). Hairy root cultures of *Brassica juncea* also showed the highest potential for phenol removal without the need for external addition of H_2O_2 (co-substrate).

It was revealed that, besides peroxidase, *B. juncea* hairy roots also produce H_2O_2 (Singh et al. 2006). Coniglio and his colleagues showed that roots were able to remove phenol concentrations up to 500 mg/L in the presence of H_2O_2, reaching high removal efficiency (Coniglio et al. 2008). *B. napus* hairy root cultures removed the contaminant with high efficiency (100–80 % for phenol solutions containing 10–250 mg/L, respectively) (González et al. 2012). Peroxidase genes (*tpx1* and *tpx2*) overexpressed in *Nicotiana tabacum* hairy roots showed higher phenol removal efficiency than wild type hairy roots after 120 h of phenol treatment at the expense of endogenous H_2O_2 (Alderete et al. 2011). Rapeseed and tomato hairy roots were used to remove phenol efficiently (100–250 mg/L) from aqueous solutions; removal efficiencies were 95–80 % and 60–70 % for rapeseed and tomato hairy root, respectively.

Addition of polyethylene glycol (PEG-3350) to the reaction medium significantly enhanced removal efficiency of rapeseed hairy root, reaching values of 98–88 % (González et al. 2008; Paisio et al. 2010). 2,4-Dichlorophenol (2, 4-DCP) is harmful for aquatic life and human health, thus there have been many attempts to remove it ysing innocuous technologies. Tobacco hairy root cultures removed 2,4-DCP in a short time and with high efficiency (98 %, 88 % and 83 %) for solutions initially containing 250, 500 and 1,000 mg/L, respectively. The highest efficiency for 2,4-DCP (500 mg/L) removal was reached at 60 min and using 10 mM H_2O_2. Moreover, hairy roots could be re-used, almost for three consecutive cycles. The diminution of pH and the increase of chloride ions in post-removal solutions suggested that 2,4-DCP dehalogenation was mediated by peroxidases (Talano et al. 2010).

Root remediation has been recognized as a cheap and eco-friendly technology that could be used for the remediation of other organic pollutants other than phenol and its chlorinated derivatives. Hairy root cultures of *Helianthus annuus* (sunflower) catalyzed the rapid disappearance of tetracycline (TC) and oxytetracycline (OTC) from aqueous media, which suggests that roots have potential for phytoremediation of these two antibiotics in vivo (Gujarathi et al. 2005). Earlier studies have demonstrated rapid dye degrading abilities in *Brassica juncea* as compared with other plants (Ghodake et al. 2009), where they mediated 79 % decolorization of textile effluent. Dye decolorization using *Brassica juncea* is associated with the involvement of intracellular laccase (Ghodake et al. 2009). Hairy roots of *Tagetes patula* L. (Marigold) were able to remove Reactive Red 198 concentrations up to 110 mg/L and could be used successively for at least five consecutive decolorization cycles. GC–MS analysis showed that Reactive Red 198 was degraded into non-toxic metabolites such as 2-aminonaphthol, p-aminovinylsulfone ethyl disulfate and 1-aminotriazine, 3-pyridine sulfonic acid (Patil et al. 2009). Hairy root cultures of *B. juncea* showed 92 % decolorization of Methyl orange within 4 days. Of the different redox mediators that were used to achieve enhanced decolorization, 2,20-azinobis, 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was found to be the most efficient (Telke et al. 2011).

DDT (1,1,1-trichloro-2,2-bis-(40-chlorophenyl) ethane) has been used widely for pest control because of its cost effectiveness, broad spectrum activity, high residual biological activity, and ease of formulation. However, exposure to DDT results in increased risk of cancer and endocrine disruption; and the environmental persistence and water insolubility of this insecticide finally led to a worldwide ban on its use (Turusov et al. 2002). Considering the potential negative effects, it is necessary to address the environmental persistence of this insecticide and to look for effective methods of remediation. Previous investigations have demonstrated that hairy roots derived from different plant species could be used for the treatment of several pesticide contaminants such as DDT (Suresh et al. 2005), and nitroaromatic compounds such as 2,4-dinitrotoluene, 2,4,6-trinitrotoluene (TNT) and aminotoluenes (Nepovim et al. 2004). Hairy root cultures of *Cichorium intybus* and *Brassica juncea* were used for their ability to uptake and degrade DDT. After 24 h of ^{14}C DDT treatment, only 12–13 % of the total applied radioactivity was detected in the culture medium, indicating the efficient uptake of DDT by the hairy roots (Suresh et al. 2005).

Catharanthus roseus hairy root cultures were able to degrade the explosive TNT to further metabolites (Hughs et al. 1997). Although our basic knowledge of the degradation of organic pollutants by plants lags far behind that of animals and bacteria, plants can transform and mineralize a wide variety of complex organics. These endogenous activities result from the ability of plants to synthesize, rearrange, and detoxify the most complex array of biochemicals and biopolymers of any living organisms, including complex carbohydrates such as cellulose and lignin.

Hairy Root Remediation of Heavy Metals

Since the industrial revolution, anthropogenic impacts have caused the release of more and more hazardous heavy metals into the environment. Unlike other organic pollutants, hazardous heavy metals are indestructible, as they cannot be degraded chemically or biologically. Even worse, some heavy metals can concentrate in the food chain and eventually accumulate in the human body because we are at the top of the food chain (Wu et al. 2010). The exploitation of plants to remove toxic heavy metals from the environment is currently of considerable commercial interest. Methods for

phytoremediation depend on the ability of plants to accumulate large quantities of metals in their tissues. In some cases, hyperaccumulation has been linked to the efficient transport of metals across the plasma membrane or into the vacuole of plant cells (Boominathan and Doran 2003a). Of particular interest for phytoremediation are the approximately 400 plant species known to hyperaccumulate heavy metals (Brooks et al. 1998).

Genetically transformed hairy root cultures have been established for a range of plant species, and applied in studies of the growth and accumulation of heavy metals. Cadmium (Cd) is a significant heavy metal pollutant from various anthropogenic activities, including use of fertilizers, sewage sludge, and compost, and from the mining, smelting, and electroplating industries. Many studies have demonstrated that Cd taken up by plants accumulates at higher concentrations in the roots than in the leaves, including in hyperaccumulators (Seregin and Ivanov 2001).

Thlaspi caerulescens is a recognized hyperaccumulator of metals. Cd levels in live *T. caerulescens* roots were 1.5- to 1.7-fold those in hairy roots of non-hyperaccumulator species exposed to the same Cd concentration (Nedelkoska and Doran 2000). *Thlaspi caerulescens* roots were found to grow at Cd concentrations of up to 890 μM, whereas non hyperaccumulator roots did not survive 178 μM. In the absence of Cd, endogenous activities of catalase were two to three orders of magnitude higher in *T. caerulescens* than in *N. tabacum* (non Cd hyperaccumulator). *T. caerulescens* roots also contained significantly higher endogenous superoxide dismutase activity and glutathione concentrations. These results showed that superior antioxidative defenses, particularly catalase activity, may play an important role in the hyperaccumulator phenotype of *T. caerulescens* (Boominathan and Doran 2003a). However, hairy roots of *T. caerulescens* contained high constitutive levels of citric, malic and malonic acids. Growth of *T. caerulescens* hairy root was severely reduced in the presence of diethylstilbestrol (DES)—an inhibitor of plasma membrane H^+-ATPase. The mechanisms of Cd tolerance and hyperaccumulation in *T. caerulescens* hairy roots are capable of withstanding the effects of plasma membrane depolarization and organic acids (Boominathan and Doran 2003b).

The mechanism of nickel (Ni) tolerance and hyperaccumulation in *Alyssum bertolonii* hairy roots is also associated with organic acids (Boominathan and Doran 2003b). *A. bertolonii* hairy root cultures accumulated the highest Ni contents in the biomass after exposure to 20 ppM Ni for up to 9 h. Ni uptake was relatively slow, with 5–7 h being required to achieve equilibrium conditions, suggesting the involvement of intracellular processes in Ni accumulation and/or detoxification. Similar Cu levels were accumulated by *Hyptis capitata* hairy roots. The initial uptake of Cu by hairy roots of *H. capitata* species occurs mainly by ion-exchange at the cell walls. In contrast, Ni uptake by hairy roots of *A. bertolonii* is slower and biphasic, consistent with the involvement of intracellular processes in Ni accumulation (Nedelkoska and Doran 2000). Furthermore, regenerated plants of *Alyssum tenium* were much more tolerant of Ni and capable of accumulating higher Ni concentrations than hairy roots of this species (Nedelkoska and Doran 2001). Due to their highly branched nature, transformed roots have a large surface area in comparison with control roots. Transformed hairy roots of *Brassica juncea* were able to translocate 100 % of the uranium from the solution to the roots at 1,000 μM uranium, while *Chenopodium amaranticolor* was restricted to 500 μM concentrations (Eapen et al. 2003). The results indicated that hairy roots of *B. juncea* and *C. amaranticolor* could remove uranium from the aqueous solution over a period of 10 days.

Heavy metals make a significant contribution to environmental pollution as a result of human activities such as mining, melting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping, and military operations. Some heavy metals, e.g., Mn, Fe, Cu, Zn, Mo and Ni, are essential or beneficial micronutrients for microorganisms, plants and animals. However, at high concentrations, all heavy metals have strong toxic effects and are an environmental threat. Hairy root cultures are a convenient experimental device for assessing the capacity and mechanisms of heavy metal uptake by plant species with potential for phytoremediation.

Arbuscular Mycorrhizal Fungi-Assisted Hairy Root Remediation

Although most research on endophytes that assist phytoremediation has focused on bacteria, the arbuscular mycorrhizas (AMs) are also involved in uptake of elements into plants (Fig. 2). Mycorrhizas are fungi that associate intimately with plant roots, increasing uptake of nutrients, especially phosphorus. AM fungi (AMF) can increase arsenic uptake in the hyperaccumulating fern *Pteris vittata* (Trotta et al. 2006). It is possible that mycorrhizas increase the uptake of arsenate, although the root arsenic concentration was low. Reduction of root growth is the first macroscopic symptom of Cd toxicity to plants. Plant resistance to Cd excess in soils can be achieved by mechanisms contributing either to the avoidance or to the tolerance of internal Cd stress. Among other factors, the symbiosis with AMF has been suggested to confer benefit to plants growing in soils with toxic Cd concentrations, though its contribution to plant resistance and the underlying mechanisms are as yet unclear (Meharg and Cairney 2000). Carrot hairy root growth was inhibited by higher Cd levels (4 mg/L). The inhibition was, however, significantly lower in treatments inoculated with AMF *Glomus intraradices* compared to the non-inoculated

control. These results showed that AMF *G. intraradice* can alleviate Cd-induced growth inhibition to carrot hairy roots (Janoušková and Vosátka 2005). Overexpression of *tpx1* in tobacco hairy roots colonized with an AMF has an efficient protective effect against phenol-induced oxidative damage (Ibáñez et al. 2011). The plant–AMF partnership could be used to enhance phytoremediation of contaminated substrates and to reduce the concentration of toxic compounds. These results clearly demonstrate the utility of using inoculations of AMFs to increase phytoremediation potential.

Concluding Remarks

Most studies of hyperaccumulation have been carried out using whole plants grown either in soil or hydroponically. For investigation of the mechanisms and metabolic responses of hyperaccumulators rather than their agronomic characteristics, there are advantages associated with alternative plant cultivation systems. In vitro culture of organs such as roots allows indefinite propagation and experimentation using tissues derived from the same plant, thus avoiding the effects of variability between individual specimens. Hairy root cultures, induced by *Agrobacterium rhizogenes* infection, have been established from a wide variety of plant species. Hairy root cultures accumulate phytochemicals to levels comparable to those of intact plants and are usually stable in their biosynthetic capacity (Zhou et al. 2011). Currently, hairy roots remediation methods have been investigated mainly in laboratory studies under well-defined conditions. Further full-scale demonstrations are required and cost-effectiveness analysis should be conducted to assess the applicability. However, successful phytoremediation may also require a more complex system-wide approach that combines many of these single methodologies.

Practical applications of hairy root remediation will require the analysis of the following topics: (1) further elucidation of the molecular mechanisms of pollutant tolerance and accumulation, particularly where and how pollutants are translocated and stored in plants or hairy roots. The emerging 'omic' technologies to identify the molecular changes in plant cells exposed to pollutants will expand knowledge and help in selection of suitable genes for incorporation into plants to improve their detoxification potential; one such example is the glutamyl cysteine synthetase gene (Sengupta et al. 2012). Since a great deal of sequence information from various organisms is now available, serial analysis of gene expression and microarray approaches can be used to identify genes directly involved in pollutant metabolism. Furthermore, environmental metagenomic data from soil and sea can be a useful source of genes. Using genetic and biochemical methods, it should be possible to clone the genes involved in remediation of both types of pollutants, and combine them in transgenic plants or hairy roots. (2) In addition, the combination of plant hairy roots to remove or degrade toxic pollutants, and rhizospheric microorganisms to enhance the availability of hydrophobic compounds, could break down many types of toxic foreign chemicals. Appropriate use of transgenic plant hairy roots and bacteria in the rhizosphere could provide a reliable means for enhancing hairy root remediation of contaminated environments and may overcome the current limitations such as detoxification and absorption efficiency of hairy root remediation. (3) In particular, more quantitative data from mass-balance studies are needed to determine the rate-limiting steps in the hairy root remediation of organic pollutants. Once the rate-limiting steps in uptake, transport, or transformation have been identified, more informed construction of transgenic plant hairy roots expressing plant, animal, or bacterial genes will result in dramatic improvements in hairy roots remediation capabilities. Although many challenges still remain for industrial applications of hairy root cultures, the immediate future of this diverse system, especially for laboratory scientists, is still promising and abound with possibilities.

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References

Alderete LGS, Agostini E, Medina MI (2011) Antioxidant response of tobacco (*Nicotiana tabacum*) hairy roots after phenol treatment. Plant Physiol Biochem 49:1020–1028

Bhadra R, Wayment DG, Hughes JB, Shanks JV (1999) Confirmation of conjugation processes during TNT metabolism by axenic plant roots. Environ Sci Technol 33:446–452

Bizily SP, Rugh CL, Meagher RB (2000) Phytodetoxification of hazardous organomercurials by genetically engineered plants. Nat Biotechnol 18:213–217

Boominathan R, Doran PM (2003a) Cadmium tolerance and antioxidative defenses in hairy roots of the cadmium hyperaccumulator, *Thlaspi caerulescens*. Biotechnol Bioeng 83:158–167

Boominathan R, Doran PM (2003b) Organic acid complexation, heavy metal distribution and the effect of ATPase inhibition in hairy roots of hyperaccumulator plant species. J Biotechnol 101:131–146

Brooks RR, Chambers MF, Nicks LJ, Robinson BH (1998) Phytomining. Trends Plant Sci 3:359–362

Chang YH, Li H, Cong Y, Lin J, Sheng BL (2012) Characterization and expression of a phytochelatin synthase gene in Birch-leaf Pear (*Pyrus betulaefolia* Bunge). Plant Mol Biol Rep. doi:10.1007/s11105-012-0447-1

Coniglio MS, Busto VD, González PS, Medina MI, Milrad S, Agostini E (2008) Application of *Brassica napus* hairy root cultures for phenol removal from aqueous solutions. Chemosphere 72:1035–1042

De la Fuente JM, Ramírez-Rodríguez V, Cabrera-Ponce JL, Herrera-Estrella L (1997) Aluminum tolerance in transgenic plants by alteration of citrate synthesis. Science 276:1566–1568

Doty SL (2008) Enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179:318–333

Dushenkof S, Vasudev D, Kapulnik Y, Gleba D, Fleisher D, Ting KC, Ensley B (1997) Removal of uranium from water using terrestrial plants. Environ Sci Technol 31:3468–3474

Eapen S, Souza SFD (2005) Prospects of genetic engineering of plants for phytoremediation of toxic metals. Biotechnol Adv 23:97–114

Eapen S, Suseelan KN, Tivarekar S, Kotwal SA, Mitra R (2003) Potential for rhizofiltration of uranium using hairy root cultures of *Brassica juncea* and *Chenopodium amaranticolor*. Environ Res 91:127–133

Eng BH, Guerinot ML, Eide D, Saier MH Jr (1998) Sequence analyses and phylogenetic characterization of the ZIP family of metal ion transport proteins. J Membr Biol 166:1–7

Ghodake G, Telke A, Jadhav J, Govindwar S (2009) Potential of *Brassica juncea* in order to treat textile effluent contaminated sites. Int J Phytoremed 11:297–312

González PS, Capozucca CE, Tigierm HA, Milrad SR, Agostini E (2006) Phytoremediation of phenol from wastewater, by peroxidases of tomato hairy root cultures. Enzyme Microb Technol 39:647–653

González PS, Agostini E, Milrad SR (2008) Comparison of the removal of 2,4-dichlorophenol and phenol from polluted water, by peroxidases from tomato hairy roots, and protective effect of polyethylene glycol. Chemosphere 70:982–989

González PS, Maglione GA, Giordana M, Paisio CE, Talano MA, Agostini E (2012) Evaluation of phenol detoxification by *Brassica napus* hairy roots, using *Allium cepa* test. Environ Sci Pollut Res 19:482–491. doi:10.1007/s11356-011-0581-6

Guerinot ML, Eide D (1999) Zeroing in on zinc uptake in yeast and plants. Curr Opin Plant Biol 2:244–249

Gujarathi NP, Haney BJ, Park HJ, Wickramasinghe SR, Linden JC (2005) Hairy roots of *Helianthus annuus*: a model system to study phytoremediation of tetracycline and oxytetracycline. Biotechnol Prog 21:775–780

Heaton ACP, Rugh CL, Wang N-J, Meagher RB (1998) Phytoremediation of mercury and methylmercury polluted soils using genetically engineered plants. J Soil Contam 7:497–509

Hughs JB, Shanks J, Vanderford M, Lauritzen J, Bhadra R (1997) Transformation of TNT by aquatic plants and plant tissue cultures. Environ Sci Technol 31:266–271

Ibáñez SG, Medina MI, Agostini E (2011) Phenol tolerance, changes of antioxidative enzymes and cellular damage in transgenic tobacco hairy roots colonized by arbuscular mycorrhizal fungi. Chemosphere 83:700–705

Janoušková M, Vosátka M (2005) Response to cadmium of *Daucus carota* hairy roots dual cultures with *Glomus intraradices* or *Gigaspora margarita*. Mycorrhiza 15:217–224

Kang S, Ajjappala H, Seo HH, Sim JS, Yoon SH, Koo BS, Kim YH, Lee S, Hahn BS (2011) Expression of the human tissue-plasminogen activator in hairy roots of Oriental Melon (*Cucumis melo*). Plant Mol Biol Rep 29:919–926

Krämer U, Chardonnens AN (2001) The use of transgenic plants in the bioremediation of soils contaminated with trace elements. Appl Microbiol Biotechnol 55:661–672

Lasat MM, Baker AJM, Kochian LY (1996) Physiological characterization of root An^{2+} absorption and translocation to shoots in Zn hyperaccumulator and non-accumulator species of *Thlaspi*. Plant Physiol 112:1715–1722

Lu YP, Li ZS, Drozdowicz YM, Hortensteiner S, Martinoia E, Rea PA (1998) AtMRP2, an *Arabidopsis* ATP binding cassette transporter able to transport glutathione S-conjugates and chlorophyll catabolites: functional comparisons with AtMRP1. Plant Cell 10:267–282

Meaghe RB (2000) Phytoremediation of toxic elemental and organic pollutants. Curr Opin Plant Biol 3:153–162

Meharg AA, Cairney JWG (2000) Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. Adv Ecol Res 30:69–112

Miland E, Smyth MR, Fágáin C (1996) Phenol removal by modified peroxidases. J Chem Tech Biotechnol 67:227–236

Nedelkoska TV, Doran PM (2000) Hyperaccumulation of cadmium by hairy roots of *Thlaspi caerulescens*. Biotechnol Bioeng 67:607–615

Nedelkoska TV, Doran PM (2001) Hyperaccumulation of nickel by hairy roots of *Alyssum* species: comparison with whole regenerated plants. Biotechnol Prog 17:752–759

Nepovim A, Podlipna R, Soudek P, Schroder P, Vanek T (2004) Effects of heavy metals and nitroaromatic compounds on horseradish glutathione S-transferase and peroxidase. Chemosphere 57:1007–1015

Oller ALW, Agostini E, Talano MA, Capozucca C, Milrad SR, Tigier HA, Medina MI (2005) Overexpression of a basic peroxidase in transgenic tomato (*Lycopersicon esculentum* Mill. cv. Pera) hairy roots increases phytoremediation of phenol. Plant Sci 169:1102–1111

Paisio CE, González PS, Gerbaudo A, Bertuzzi ML, Agostini E (2010) Toxicity of phenol solutions treated with rapeseed and tomato hairy roots. Desalination 263:23–28

Patil P, Desai N, Govindwar S, Jadhav JP, Bapat V (2009) Degradation analysis of Reactive Red 198 by hairy roots of *Tagetes patula* L. (Marigold). Planta 230:725–735

Santos de Araujo B, Charlwood BV, Pletsch M (2002) Tolerance and metabolism of phenol and chloroderivatives by hairy root cultures of *Daucus carota* L. Environ Pollut 117:329–335

Sengupta D, Ramesh G, Mudalkar S, Kumar KRR, Kirti PB, Reddy AR (2012) Molecular cloning and characterization of γ-glutamyl cysteine synthetase (*VrγECS*) from roots of *Vigna radiata* (L.) Wilczek under progressive drought stress and recovery. Plant Mol Biol Rep. doi:10.1007/s11105-011-0398-y

Seregin IV, Ivanov VB (2001) Physiological aspects of cadmium and lead toxic effects on higher plants. Russ J Plant Physiol 48:523–544

Singh S, Melo JS, Eapen S, D'Souza SF (2006) Phenol removal using *Brassica juncea* hairy roots: role of inherent peroxidase and H_2O_2. J Biotechnol 123:43–49

Suresh B, Bais HP, Raghavarao SMS, Ravishankar GA, Ghildyal NP (2005) Comparative evaluation of bioreactor design using *Tagetes patula* L. hairy roots as a model system. Process Biochem 40:1509–1515

Talano MA, Agostini E, Medina MI, De Forchetti SM, Tigier HA (2003) Tomato (*Lycopersicon esculentum* cv. Pera) hairy root cultures characterization and changes in peroxidase activity under NaCl treatment. In Vitro Cell Dev Biol Plant 39:354–359

Talano MA, Agostini E, Medina MI, Reinoso H, Tordable MC, Tigier HA, de Forchetti SM (2006) Changes in ligno-suberization of cell walls of tomato hairy roots produced by salt treatment: the relationship with the release of a basic peroxidase. J Plant Physiol 163:740–749

Talano MA, Frontera S, González P, Medina MI, Agostini E (2010) Removal of 2,4-diclorophenol from aqueous solutions using tobacco hairy root cultures. J Hazard Mater 176:784–791

Telke AA, Kagalkar AN, Jagtap UB, Desai NS, Bapat VA, Govindwar SP (2011) Biochemical characterization of laccase from hairy root culture of *Brassica juncea* L. and role of redox mediators to enhance its potential for the decolorization of textile dyes. Planta. doi:10.1007/s00425-011-1469-x

Trotta A, Falaschi P, Cornara L, Minganti V, Fusconi A, Drava G, Berta G (2006) Arbuscular mycorrhizae increase the arsenic translocation factor in the as hyperaccumulating fern *Pteris vittata* L. Chemosphere 65:74–81

Turusov V, Rakitsky V, Tomatis L (2002) Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. Environ Health Perspect 110:125–128

Wu J, Taylor KE, Bewtra JK, Biswas N (1993) Optimization of the reaction conditions for enzymatic removal of phenol from wastewater in the presence of polyethylene glycol. Water Res 27:1707–1714

Wu G, Kang HB, Zhang XY, Shao HB, Chu LY, Ruan CJ (2010) A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. J Hazard Mater 174:1–8

Zhou ML, Zhu XM, ShaoJR TYX, Wu YM (2011) Production and metabolic engineering of bioactive substances in plant hairy root culture. Appl Microbiol Biotechnol 90:1229–1239