

Progress of Phytoremediation: Focus on New Plant and Molecular Mechanism

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Abstract

Phytoremediation use plants to remove pollutants from the environment and render them harmless. It is an economically feasible and sustainable ways regarding to the remediation of our environment without introducing any new contaminants. Considering the vast diversity of pollutants and the different kinds of environmental conditions are two most important aspects that plants used for the remediation have to overcome, it is get more and more feasible for people looking for and developing new plants. Understanding the molecular mechanism of a plant's responses to pollutant stress is the key to creating new plant species for this purpose through genetic engineering. In this review, we focus on the progress on searching for new plant species suitable for phytoremediation and the molecular mechanisms of their responses to pollutant stress. At the end, we propose some suggestions on how we can encourage the use of phytoremediation in the future.

Introduction

Since the beginning of the Industrial Revolution, anthropogenic activities have been generating large amounts of non-biodegradable compounds that can be separated into organic and inorganic compounds. Organic pollutants mainly include chlorinated solvents like trichloroethylene, petroleum hydrocarbons such as benzene, toluene and xylene, explosives like Trinitrotoluene (TNT), Polyaromatic hydrocarbons (PTHs), and pesticides like atrazine and bentazon. In the case of inorganic compounds, heavy metals, such as lead, zinc and cadmium, and radioactive elements, such as uranium, are more commonplace [1]. Most of them were released into the environment, causing severe destruction to the ecosystem and, being non-biodegradable, caused adverse effects on human health due to bioaccumulation.

In most of case, the only way to restore the environment is removing the pollutants. Previous methods used to remediate the environment were based on the physico-chemical theory, like volatilization, vitrification, excavation, soil washing, soil incineration, chemical extraction, solidification, and landfills. Although these methods are effective in numerous interventions, they are often expensive, invasive, and are not suitable for large-area application. In this context, the concept phytoextraction was first proposed by Chaney [2], and subsequently quickly sparking the interest of many botanists and making phytoremediation a popular field [3]. In comparison to the methods previously used, phytoremediation is cost effective and suitable for very large areas of land [4]. In addition, it can also produce biomass to reduce the pressure of food production caused by energy crops. Phytoremediation is currently divided into the following areas: (1) phytoextraction use hyperaccumulators to remove metals or organics from soil by concentrating them in above soil tissue, (2) phytodegradation use of plants and their associated microorganisms to degrade organic pollutants, (3) rhizofiltration use of plant roots



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to absorb and adsorb pollutants, mainly toxic heavy metals, from water and aqueous waste streams, (4) phytostabilization use plants to reduce the bioavailability of pollutants in the environment, and (5) phytovolatilization use plants to volatilize pollutants and remove them from air. Based on the phytoremediation techniques listed above, we can see that plants have abilities of cleaning waste elements in different ways, and phytoaccumulation is the most widely studied topic. Plants that can grow on metalliferous soils without suffering phytotoxic effects and accumulate extraordinarily high amounts of heavy metals in the aerial organs are described as hyperaccumulators. To date, there are more than 450 hyperaccumulation species, accounting only for less than 0.2% of all known species [5]. Because of the diversity of pollutants and environments, finding a specific plant that is able to withstand different heavy metals and organic matter in different kinds of environmental conditions is very important. In addition, cloning of genes responsible for those qualities and uncovering the molecular mechanisms behind them are the foundation of genetically engineering a plant that will be more effective in cleaning up the environment. During the past decades, phytoremediation technologies developed very fast and many new phytoremediation plants emerged. In the following sections, we will summarize the progress on searching for new phytoremediation plant species and their molecular mechanisms study progress.

New Phytoremediation Plants Seeking

For heavy metals

Heavy metals are main culprits polluting the environment and are caused by a number of human activities, such as mining, smelting, electroplating, use of pesticides, sludge dumping, and (phosphate) fertilizers as well as biosolids in agriculture [6]. Heavy metals are the most widely studied among all the pollutants. Here, we summarize some of studies carried out regarding phytoremediation of heavy metals.

Hydrilla verticillata (L.f.) Royle is a submerged macrophyte

widely distributed throughout the world. Xue and Yan [7] showed that the shoot of *H. verticillata* has the ability to accumulate arsenic (AS) and its potential for arsenic phytofiltration. To provide some insight on the possibility of using serpentine adapted plants for phytoextraction of cadmium, Barzanti et al. [8] investigated variations in cadmium tolerance, accumulation and translocation in three Alyssum plants, and the results indicated that the serpentine adapted population of *Alyssum montanum* showed statistically higher cadmium tolerance and accumulation than *A. bertolonii* and the ones not adapted to serpentine soil. The effectiveness of two aquatic macrophytes, *Ceratophyllum demersum* and *Lemna gibba*, removing two toxic heavy metals Pb and Cr were investigated [9]. It showed that *L. gibba* was more efficient at the removal of selected heavy metals than *C. demersum*. *L. gibba* can also accumulate heavy metals without the production of toxins. *Brachiaria mutica* (Forssk) Stapf was found behaving luxuriant growth with massive fibrous roots when grown in Cr contaminated soils (11,170 mg/kg dry soil) [10]. These results indicated that paragrass could be used to remediate chromium contaminated soils *in situ* as it showed rapid growth even with a high concentration of Cr present. Six tree species' potentials for phytoremediation abilities of Pb in sand tailings were tested and the result showed that *Acacia mangium*, with the addition of organic fertilizer was the best option [11]. More recently, *Nopalea cochenillifera* was proved being a potential chromium (VI) hyperaccumulator plant [12,13] assessed the potential for phytoremediation of heavy metals (Ni, Pb, and Zn) of three endemic Mediterranean plant species, *Atriplex halimus*, *Portulaca oleracea* and *Medicago lupulina*. Due to its high biomass production and the relatively high roots metal contents, *A. halimus* and *M. lupulina* could potentially be used in phytoremediation and phytostabilization. The potential of kenaf (*Hibiscus cannabinus* L.) and corn (*Zea mays* L.) for phytoremediation of dredging sludge contaminated by trace metals, Tolerance and bioaccumulation factors were tested, and the results showed that both species could be used in phytoremediation [14]. Pratas et al. [15] assessed the phytoremediation potential of flora that is tolerant to heavy metal in the contaminated soils of an abandoned Pb mine in Central Portugal. They found several plants that had the highest uptake of metals, including *Cistus salvifolius* (548 mg Pb kg⁻¹), *Digitalis purpurea* (1017 mg Zn kg⁻¹) and 4450 mg Fe kg⁻¹), *Mentha suaveolens* (1.9 mg Ag kg⁻¹), and *Ruscus ulmifolius* (1 mg Ag kg⁻¹). *Ricans communes* L. (castor oil plant) is an energy plant, which is well suited to cope with the local toxic conditions and can be useful for decreasing metal bioavailability, dispersion and human health risks [16]. Recently, *Wolffia globosa* was found to be a strong Cd accumulator and has great potential for Cd phytoremediation [17]. On top of that, the fresh fronds also showed impressive As extracting ability. Hence, there is a possibility that it can be used in fresh aquatic environments co-contaminated by low-levels of both Cd and As.

For other pollutants

Besides from heavy metals, there are also progresses in the phytoremediation of other pollutants such as polychlorinated biphenyl (PCB), benzo[a]pyrene (B[a] P), and Tetracycline. Ficko et al. [18] investigated the effects of plant age, contaminant characteristics, and species-specific properties on PCB uptake and accumulation patterns in plant tissues of the three perennial weed species [L. (*ox-eye daisy*), L. (*curly dock*), and L. (*Canada goldenrod*)]. Their results showed that for each of these weed species, shoot contaminant concentrations and

total biomass are dependent on plant age and life cycle (vegetative and reproductive stages), which are in relation with the total amount of PCBs phytoextracted on a per-plant basis. Sun et al. [19] showed that the French marigold (*T. patula*) might be useful for phytoremediation of B[a] P and B[a] P-Cd contaminated sites. The common vetch is able to tolerate and remove high phenol concentrations and avoid serious phytotoxic effects, indicating that *V. sativa* could be considered as an interesting tool in the field of phytoremediation. The presence of veterinary and human antibiotics in soil and surface water is also an emerging environmental concern. Datta et al. [20] evaluated the potential of using vetiver grass as a phytoremediation agent in removing Tetracycline (TC) from aqueous media. The data collected is encouraging and is expected to aid in the development of a cost-effective, *in-situ* phytoremediation technique to remove antibiotics consisting of TC groups from wastewater. Ma et al. [21] conducted a field experiment to study the removal of phthalic acid esters (PAEs) by legume (*alfalfa*, *Medicago sativa* L.) grass (*perennial ryegrass*, *Lolium perenne* L. and tall fescue, *Festuca arundinacea*) intercropping in e-waste contaminated agricultural soils in China. Their results showed that phytoremediation with alfalfa was effective in both monoculture and intercropping and phytoremediation may have the potential to remove PAEs from contaminated soils. Saiyood et al. [22] found that an evergreen mangrove tree, *B. ruguiera* gymnorhiza, is tolerant to bisphenol A (BPA) and has the capability to remove BPA. Souza et al. [23] showed that *M. aquaticum* can reduce oxygen demand (COD), biochemical oxygen demand (BOD), and total phosphorus (TP) in 15 days, and ammoniacal nitrogen (AN) as well as total Kjeldahl nitrogen (TKN) in 30 days, indicating that it is a potential plant that can be used in phytoremediation of polluted water.

Molecular Mechanism of Phytoremediation

Uncovering the underlying molecular mechanisms in plants, especially those capable of hyperaccumulation, will provide further insights for engineering plants for phytoremediation in the future. Heavy metals are the main group of pollutants and progress in the molecular mechanism of plant stress response to heavy metals has been made, especially in herbaceous plants such as *Arabidopsis thaliana*, *A. halleri* and *T. caerulea* [24-27]. High-throughput technologies, in particular microarray, have allowed the complexity of plant stress response to be tackled. Much work has been reported recently in these filed. Here, we reviewed the progresses since 2009. For more information, readers can refer to the reviews by Verbruggen et al. [24], Thapa et al. [25], Claire-Lise and Nathalie [26] and other related papers.

Arabidopsis thaliana

A. thaliana has always played a very important role in uncovering the molecular mechanism of plant response to pollutants as its genome information is available and it can be easily mutated. Three *Arabidopsis* genes, oxophytodienoate reductases 1 (OPR1), OPR2, and OPR3, were found to be up-regulated by exposure to TNT [28]. Subsequent biochemical characterization revealed that two of the three OPR1 lines and all of the OPR2-overexpressing lines exhibited enhanced tolerance to TNT. Rao et al. [29] identified the potential target gene in *A. thaliana* for phytoremediation and phytosensing of chemical contaminants, RDX and TNT, by microarray analysis. Genes that were differentially expressed included oxidoreductases, cytochrome P450s, transferases, transporters, and several unknown expressed proteins. Two transcription factors bZIP19 and bZIP23

were found to be able to regulate the adaptation to zinc deficiency and zinc homeostasis in plants [30]. To detect the potential genes that are related to the sensing mechanism and metabolism of toluene, Gao et al. [31] conducted a microarray analysis on the seedlings grown on toluene-containing media. The results show a coordinated induction and suppression of 202 and 67 toluene-responsive genes respectively, include genes encoding cytochrome P450s, glucosyl transferases, and transporters. Pineau et al. [32] revealed cross-talk between Fe homeostasis and Zn tolerance in *A. thaliana* by analyzing natural variation at the FRD3 MATE transporter locus.

Populus

Gaudet et al. [33] compared the physiological and molecular response to cadmium stress in two *Populus nigra* L. genotypes originating from contrasting environments in northern (genotype 58-861) and southern (genotype Poli) Italy. Their results showed that the later was markedly more tolerant to Cd stress and the glutathione pathway was also involved in the differential Cd tolerance of the two genotypes. Luo et al. [34] conducted the transcript analysis of *Populus × canescens* response to cadmium. They found that about 48% of the differentially regulated transcripts formed a co-regulation network in which 43 hub genes played a central role in cross talk among distinct biological processes. This enhanced our understanding about the molecular mechanism of woody plant response to heavy metal.

The correlation analysis of SNP diversity with the phenotypic response to exposure to cadmium in *Populus* spp was conducted, and a positive correlation was established between genetic variation, cadmium accumulation, and its bioconcentration in the root [35]. The quantitative trait loci (QTL) and candidate genes for cadmium tolerance in *Populus* were identified [36]. Functional characterization of these candidate genes should enhance our understanding of Cd metabolism and transport and phytoremediation capabilities of *Populus*.

Brassica juncea

Brassica juncea is a promising plant species that can be used for phytoremediation of heavy metals. *B. juncea* root proteome in response to cadmium exposure was analyzed, and the enzymes such as peptide methionine sulfoxide reductase, and 2-nitropropane dioxygenase in alternative redox-regulation mechanisms, as well as O-acetylserine sulfhydrylase, glutathione-S-transferase, and glutathione-conjugate membrane transporter were essential players in the Cd hyperaccumulation and tolerance of *B. juncea* [37]. The transcripts levels of two *B. juncea* cation-efflux family proteins, BjCET3 and BjCET4 could be substantially increased by the introduction of Zn, Cd, NaCl or PEG, suggesting that BjCET3 and BjCET4 may play roles in those stress conditions [38].

Crambe abyssinica

Crambe abyssinica (a member of Brassicaceae), a non-food, fast growing high biomass crop, is an ideal candidate for phytoremediation of heavy metal contaminated soils. Thirty eight genes involving in arsenic metabolism and detoxification were isolated successfully [39]. In response to Cr exposure in *C. abyssinica* by a PCR-Select Suppression Subtraction Hybridization approach, a total of 72 differentially expressed subtracted cDNAs were sequenced and found to represent 43 genes [40].

Other plants

Elsholtzia splendens is generally considered as a Cu-tolerant and -accumulating plant species and is a likely candidate for phytoremediation of Cu-contaminated soils. Li et al. [41] conducted proteomic analysis of copper stress response in *E. splendens* roots and leaves by two-dimensional gel electrophoresis and found that 45 protein spots were significantly changed in roots, but only 6 were changed in leaves. The identified root proteins were involved in various cellular processes such as signal transduction, regulation of transcription and translation, energy metabolism, regulation of redox homeostasis, and cell defense while the leaf proteins were mainly degraded fragments of RuBisCo and antioxidative protein. Depending on Cd and Zn uptake, several antioxidant enzymes showed significantly different activities in *Nicotiana tabacum* [42]. Although SOD and CAT were usually elevated, several other enzymes and isoforms of GST were strongly inhibited. They suggested that when planning phytoremediation of sites, mixed pollution scenarios have to be anticipated and the plants should be selected according to both their stress resistance and hyperaccumulative capacity. A broccoli (*Brassica oleracea var italica*) cDNA encoding COQ5 methyltransferase (BoCOQ5-2) in the ubiquinone biosynthetic pathway was cloned [43]. Transgenic *Arabidopsis* expressing BoCOQ5-2 volatilized three times more Se than the vector-only control plants when treated with selenite and exhibited an increased tolerance to Se.

Eleven QTLs for arsenic accumulation in maize (*Zea mays* L.) were detected [44]. In *Portulaca oleracea*, the peroxidase 2a (PoPRX2a) is potentially useful in the remediation of phenolic pollutants [45]. In Se-hyperaccumulator *Astragalus racemosus*, out of the 125 Se-responsive candidate genes identified, six of them responded to both selenate and selenite treatments. A novel gene CEJ367 was highly induced by both selenate (1,920-fold) and selenite (579-fold). These identified genes may allow us to create Se-enriched transgenic plants [46]. HvHMA2, a P (1B)-ATPase from barley, is highly conserved among cereals and functions in Zn and Cd transport [47]. *Solanum nigrum* was found to be a cadmium (Cd) accumulator, and the transcriptome analyses revealed higher expression of the genes that encoded several metal transporters as well as antioxidant-related genes, and several organic and amino acid biosynthesis/metabolism-related genes in Cd-treated *S. nigrum*, which indicated that the different responsive mechanisms of the transporter genes to Fe deficiency might be responsible for differential uptake and redistribution of metals in the two *Solanum* species [48]. A major latex-like protein is a key factor in Cucurbitaceae family crop contamination by persistent organic pollutants [48]. TaHMA2 is another gene from wheat (*Triticum aestivum* L.), which belongs to heavy metal ATPase 2 (HMA2) [50].

Conclusion and Perspectives

The environment has become more and more concerned in the fields of economics, politics, social and cultural affairs, science and technology. To enhance the potential for environmental protection and conservation, a considerable amount of researches have been done for phytoremediation. However, those studies in the field of biological applications do not provide much attention to uncertainty in the molecular principles. The phytoremediation project is still under investigation as well in methodological aspects as in concrete applications. In order to move phytoremediation forward, it is important to look for and develop new plant species with the ability to

remove contaminants from our environment. Based on our current knowledge, we can see that a lot more work needs to be done before we can fully utilize this method to clean the environment as: (1) There are still many pollutants, such as chlordane and dieldrin, that lack a suitable functional plant, and (2) it often happens that different pollutants exist together in polluted soils and waters, while most of the current research only focus on one type of contaminant.

The plants suited for phytoremediation are fast growing and is able to produce a large amount of biomass. However, many potential plants lack these characteristics. Thus, the possibility to produce new plants through transgenic methods is a good choice. There are two methods can be adopted to create a new plant that displays both these qualities. One is to increase the biomass of known plants that are already suitable for phytoremediation and the other is to improve the remediation ability of some plants with large amount of biomass, such as populus and corn. From the above reviews, we can see that there have been leaps and bounds in the latter in recent years. As for the former, we think that more can be accomplished with the development of genomics.

Considering that the growth habits are familiar to human beings and the genome information have been or are being uncovered, some crops and economic wood plants with large amount of biomass and are potential energy plants are most promising to be used to development new plants for phytoremediation. Phytoremediation is a low-cost and efficient approach for environment recovery. Although there are some successful cases of phytoremediation, more work needs to be done for it to be applicable worldwide. Finally, it should be kept in mind that phytoremediation is an interdisciplinary area of research where plant biology, microbiology, soil science, genetic engineering, and environmental modeling converge.

China is faced with increasingly severe environmental pollution, and this subject has been extensively explored. In the long run, to clean up and remediate environment is a mandatory task for Chinese government. In order to achieve these objectives, phytoremediation will become a sustainable alternative and play important roles in the remediation of the Chinese environment combined with other approaches. Genetically modified turf grass tolerant of high organic and metal pollution in soils could be used for environmental remediation. They are suitable candidates as they have extensive fibrous root systems for reaching greater areas of land. On top of that, it also allows for a larger rhizosphere microbial community. Interactions with the surrounding soil would also increase and improve contaminant removal efficiency. Secondly, these plants are extremely capable of fast regeneration, which increases their chances of survival in rough environments. Conclusively, they will be excellent for phytoremediation.

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