Advances in the Application of Plant Growth-Promoting Rhizobacteria in Phytoremediation of Heavy Metals

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1 Introduction

Rapid industrialization and modernization around the world have produced the unfortunate consequence of releasing toxic wastes to the environment. Metal pollutants are derived mainly from industrial and agricultural activities. The former includes activities such as waste disposal, chemical manufacturing, and metal pollutants from vehicle exhaust, and the latter involves activities such as the use of agrochemicals, long-term application of sewage sludge, and wastewater to agricultural soils. Such releases have adversely affected human health and have produced toxic effects on plants and the soil microorganisms associated with them. Toxic metal contaminants from wastes or other products accumulate in the agricultural

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soils to which they are applied, threaten food security, and pose health risks to living organisms by their transfer within the food chain. Once heavy metals reach the soil, they are absorbed by plants and may be taken up by animals and humans through consumption of contaminated food or drinking water. They may even be inhaled as particulate contaminants, and due to their persistent nature, they may accumulate in both plants and animals over time.

Although plants may suffer damage from excessive contact with heavy metals, they also rely on a range of transition (heavy) metals as essential micronutrients for normal growth and development. Among metals, some comprise elements that are essential for most redox reactions and are fundamental to normal cellular functions. Fe, for example, is a key component of haem proteins such as cytochromes, catalase, and Fe-S proteins (e.g., ferredoxin). Cu is an integral component of certain electron transfer proteins in photosynthesis (e.g., plastocyanin) and respiration (e.g., cytochrome *c* oxidase) processes, whereas Mn is less redox active but has a key role in photosynthesis (e.g., O_2 evolution). Zn is non-redox active but has a vital structural and/or catalytic role in many proteins and enzymes. Ni is a constituent of urease, and small quantities of Ni are essential for some plant species (Sirko and Brodzik 2000). When amounts of any of these metals are in short supply, a range of deficiency symptoms appear and growth is reduced. Notwithstanding, these transition metals, when present in excessive amounts, interfere with cellular functions, and alter normal metabolic processes to produce cellular injuries, and potentially plant death. Many target molecules in cells, whose structure/activity is inhibited, modified or enhanced by transition metals have been identified. Ochiai (1977) lists three events that generate plant toxicity by transition (heavy) metals. These are

(a) Displacing essential components in biomolecules
(b) Blocking essential biological functions of molecules
(c) Modifying enzyme/proteins, plasma membrane, and/or membrane transporters' structure/function

Cellular enzymes and proteins contain several mercapto ligands that can structurally chelate metals, and thereby cause these proteins to lose their functional property. Heavy metals also generate oxidative stress that is mediated through generation of free radicals (Seth et al. 2008). Some heavy metals directly affect biochemical and physiological processes through inhibition of photosynthesis and respiration leading to reduced growth (Vangronsveld and Clijsters 1994). Therefore, excessive heavy-metal accumulation in plants may induce toxicity by modifying essential protein structure or replacing essential elements that is manifested by chlorosis, growth impairment, browning of roots, and inactivation of photosystems, among other effects (Shaw et al. 2004; Gorhe and Paszkowski 2006).

Governmental entities and their citizens alike have come to understand that consuming contaminated food or drinking water can expose both humans and animals to toxic levels of heavy metals. Lead poisoning is not uncommon, and is perhaps is the best example of a heavy metal the effects of which are understood by people. It has been estimated that lead exposure affects more than 800,000 children between the age of 1 and 5 in the USA (Lasat 2002). To deal with known exposures, whether from point sources, contaminated sites, or spills, efforts are routinely taken to remediate and control heavy-metal pollutants in soils and elsewhere. The three major options to remediate toxic metals are through physical, chemical, or biological means. The latter approach is referred to as bioremediation. Bioremediation is defined as the application of biological processes to remove hazardous chemicals from the environment. It has obvious advantages over physicochemical remediation in that it is more cost effective, more convenient to undertake, and produces less collateral destruction of onsite substrate or indigenous flora and fauna (Timmis and Pieper 1999).

One form of bioremediation is called phytoextraction. It is emerging as a promising and cost-effective approach to remediate metal contaminated sites, and has advantages over the alternative chemical and physical remediation technology approaches (Zhuang et al. 2007). How successful phytoremediation will be at any one site depends on the extent of soil contamination, bioavailability of the metal contaminant involved, and the ability of the plant used to absorb and accumulate metals as biomass. Generally, plants with exceptionally high metal accumulating capacity often grow slowly and produce limited biomass, particularly when the metal concentration in the soil is high. However, there is a way to maximize the chances of success of phytoremediation by utilizing plant growth-promoting rhizobacteria (PGPR), which are soil microbes that inhabit the rhizosphere. When PGPR are introduced to a contaminated site, they increase the potential for plants that grow there to sequester heavy metals and to recycle nutrients, maintain soil structure, detoxify chemicals, and control diseases and pests; PGPR also decrease the toxicity of metals by changing their bioavailability in plants. The plants, in turn, provide the microorganisms with root exudates such as free amino acids, proteins, carbohydrates, alcohols, vitamins, and hormones, which are important sources of their nutrition. The rhizosphere has high concentrations of root-exuded nutrients and attracts more bacteria than does bulk soil (Han et al. 2005; Babalola 2010). These bacteria of the rhizosphere, therefore, facilitate plant growth.

PGPR serve several functions for plants. They fix atmospheric nitrogen and supply it to plants, or they synthesize siderophores that can solubilize and sequester iron from the soil and provide it to plant cells. PGPR also synthesize several different phytohormones, including auxins (IAA) and cytokinins, which enhance plant growth and solubilize minerals such as phosphorus, thereby rendering it more readily available for plant growth. Moreover, PGPR contain enzymes that modulate plant growth and development (Glick et al. 1998; Sheng and Xia 2006; Ma et al. 2009).

The concept of using green plants to extract metals is not new. The original concept has, however, been expanded to include the use of an interdisciplinary approach, in which microbes, in close association with plants, are employed to enhance the removal, immobilization, or degradation of certain metals from polluted soils. Such enhanced action results from the presence of PGPR. PGPR comprise several different genera (e.g., *Pseudomonads* and *Acinetobacter*) and enhance the phytoremediation abilities of non-hyperaccumulating maize (*Zea mays* L.) plants by increasing their growth and biomass (Lippmann et al. 1995). Although, until recently, data on microbe-assisted metal extraction have been scarce, several papers have been published on the topic during the last 5 or 6 years. The main aim we have in this chapter is to describe how the partnership between plants and associated microbes (viz., PGPR) can be exploited as a strategy to improve plant biomass production and heavy-metal removal from metal-contaminated sites.

2 Phytoremediation

Phytoremediation is an in situ biomediation process that uses green plants and the microorganisms that are associated with them to extract, sequester, or detoxify pollutants. Plants have the capacity to take up, accumulate, degrade, or eliminate metals, pesticides, solvents, crude oil, and many industrial contaminants. Phytoremediation is a clean, cost-effective, environment-friendly technology, especially for treating large and diffused areas that are contaminated. There are many successful examples where phytoremediation has been employed, and where it has been documented to work well for remediating contaminated industrial environments (Macek et al. 2000; Suresh and Ravishankar 2004). Depending on the method used and nature of the contaminant involved, phytoremediating areas where metals and other inorganic compounds exist, may utilize one of several techniques (Glick 2003; Newman and Reynolds 2004) as follows.

(a) *Phytoextraction*: also known as phytoaccumulation, removes metals by taking advantage of the unusual ability of some plants to (hyper-)absorb and accumulate or translocate metals or/metalloids, by concentrating them within the biomass. The purpose of this type of remediation is to reduce the concentration of metals in contaminated soils so that they can be used profitably for agriculture, forestry, horticulture, grazing, etc.
(b) *Phytostabilization*: also known as phytoimmobilization, utilizes plants, often in combination with soil additives, to assist in mechanically stabilizing sites for reducing pollutant transfer to other ecosystem compartments and to the food chain; the “stabilized” organic or inorganic compound is normally incorporated into plant lignin or into soil humus. The basis for phytostabilization is that metals do not degrade, so capturing them in situ is often the best alternative. This approach is particularly applicable when low-concentration, diffused, and vast areas of contamination are to be treated. Plants restrict pollutants by creating a zone around the roots where the pollutant is precipitated and stabilized. When phytostabilization is undertaken, the plants used do not absorb the targeted pollutant(s) into plant tissue.
(c) *Phytostimulation*: plant roots promote the development of rhizosphere microorganisms that are capable of degrading the contaminant, and the microbes utilize plant root exudates as a carbon source.
(d) *Phytovolatilization/rhizovolatilization*: employs metabolic capabilities of plants and the associated rhizospheric microorganisms to transform pollutants into volatile compounds that are released into the atmosphere. Some ions (i.e., of

elements of subgroups II, V, and VI of the periodic table like mercury, selenium, and arsenic) are absorbed by roots, are converted into less toxic forms, and then are released.

(e) *Phytodegradation*: organic contaminants are degraded or mineralized by specific enzymes.

(f) *Rhizofiltration*: use terrestrial plants to absorb, concentrate, and/or precipitate contaminants in the aqueous system. Rhizofiltration is also used to partially treat industrial and agricultural runoff.

Plants that can potentially accumulate large quantities of metals by natural methods have been identified, and are being studied for their use to remediate heavy-metal contaminants. These plants are called hyperaccumulators, and are often found growing in areas that have long had elevated metal concentrations in soil. Unfortunately, at high enough metal levels, even hyperaccumulating plants are slow growing and attain only a small size. Thus, high metal levels inhibit plant growth, even in plants that are capable of hyperaccumulating them. Depending upon the amount of metal at a particular site and the type of soil, even hyperaccumulating plants may require 15–20 years to remediate a contaminated site. This time frame is usually too slow for practical application. Therefore, research undertaken to find such plants should emphasize species that are fast growing and accumulate greater amounts of biomass, in addition to their being tolerant to one or more heavy metals. Moreover, the success of phytoremediation depends on the metal in the soil being in the bioavailable fraction. Hence, it is also important that researchers study the bioavailability and uptake of target metals by hyperaccumulating plants. When research is dedicated to finding optimal hyperaccumulator plants, key study goals should include both (1) evaluating the impact of metal stress on beneficial rhizospheric microbes and crops and (2) predicting the application of bioremediation technologies that could be used to clean up metals from the polluted soils.

2.1 Biological Availability of Metals in Soil

The term bioavailability is usually ill defined and rarely quantified, particularly in microbial investigations. In reality, bioavailability cannot be measured. It can only be estimated by measuring the growth of organisms, and by evaluating uptake or toxicity of the metal. As mentioned earlier, several industrial operations (e.g., smelting, mining, metal forging, manufacturing of alkaline storage batteries, and combustion of fossil fuels) release toxic metals, and agrochemicals and long-term application of sewage sludge and wastewater are used to augment agricultural production, all of which add amounts of metals to soil (Giller et al. 1989; McGrath et al. 1995; Tak et al. 2010). In soil, such metals exist in both bioavailable and non-bioavailable forms (Sposito 2000) and their mobility depends on whether they (1) precipitate in soil as positively charged ions (cations) or (2) are associated with negatively charged salt components of soil. The major reason of the low metal extracted by the plants from the soil is their low bioavailability. The bioavailability of metals from soil depends on soil factors such as cation exchange capacity (CEC), organic matter content, the content of clay minerals and hydrous metal oxides, pH, buffering capacity, redox potential, water content, and temperature (Kayser et al. 2001). In addition, plant root exudates and microbial activities in soil also influence metal bioavailability to plants (Brown et al. 1999; Traina and Laperche 1999).

Several researchers have shown the positive impact of bioaugmentation on metal bioavailability. In one such study, exchangeable Pb was increased by 113% when *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* were present (Braud et al. 2006). In another study, extractable Ni was increased by the presence of *Microbacterium arabinogalactanolyticum* (Abou-Shanab et al. 2006). The toxicity of the heavy metals within soils of high CEC is generally low, even when metal concentrations are high (Roane and Pepper 2000). However, under oxidizing and aerobic conditions, metals are usually present as soluble cationic forms, whereas under reducing or anaerobic conditions they exist as sulfide or carbonate precipitates. At low soil pH, bioavailability increases due to the presence of metals as free ionic species, and just the opposite occurs when soil pH is high (i.e., when insoluble metal mineral phosphates and carbonates are formed). The soil mobility and bioavailability of the following metals usually proceed in this order: Zn > Cu > Cd > Ni (Lena and Rao 1997). When metals coexist with other metals or contaminants the rate of absorption and accumulation into food webs, and ultimately into animal and human diets, may be accentuated.

2.2 Plant Uptake and Transport of Metals

Plants have developed mechanisms by which they can effectively absorb metals from the soil solution and transport them to other parts within the plant. Most metal-accumulating species were discovered in areas having a high metal concentration, and majority of such areas exist in tropical regions. These natural plant hyperaccumulators of metal represent diverse taxa, although the majority exist in the family Brassicaceae. For example, Indian mustard (*Brassica juncea*) rapidly concentrates Cd (II), Ni (II), Pb (II), and Sr (II) into root tissues at levels 500 times greater than the liquid medium in which they are growing (Salt et al. 1995; Salt and Kramer 1999). Uptake of metals into root cells, which is the point of entry into living tissue, is a major step in the phytoextraction process. However, for phytoextraction to be successful, the absorbed metals must be transported from root to shoot. The mechanisms by which metals are absorbed into the plant root are complex. This process involves transfer of metals from the soil solution to the root–surface interface, and then penetration through the root membranes to root cells. Metal ions cannot move freely across the cellular membrane because of their charge. Therefore, ion transport into cells must be mediated by membrane proteins that have a transport function, and these are generically referred to as transporters. These transporters possess an extracellular domain to which the ions attach just before the transport, and a transmembrane binding structure that connects extracellular and intracellular media.

This is an oversimplification, and the uptake process is actually rendered even more complex by the nature of the rhizosphere (Laurie and Manthey 1994).

Hyperaccumulator plants take metals up from soil in direct proportion to their bioavailability (Wenzel et al. 2003). Metal bioavailability to plants is often modified by the direct influence of root activity in the rhizosphere (Hinsinger et al. 2005). Bioavailability is regulated by electrochemical potential gradient that exists for each metal ion across the plasma membrane of root cells (Welch 1995). However, the exact nature of the membrane transporters that control the influx across the plasma membrane into the cytoplasm is not yet known.

2.3 Plant Mechanisms for Metal Detoxification

Although micronutrients such as Zn, Mn, Ni, and Cu are essential for plant growth and development, high intracellular concentrations of these ions can be toxic. To deal with this potential stress, common non-accumulator plants have evolved several mechanisms to control the intracellular homeostasis of ions (Lasat 2002; Seth et al. 2007). One such mechanism is to regulate ion influx. This involves stimulating transporter activity at a low level of intracellular ion supply, and inhibiting activity at high concentrations, or extruding ions from the cell interior to the external solution (Pollard et al. 2002). Metal hyperaccumulator species are capable of accumulating metals up to multiple thousands of ppm, and are equipped with detoxification mechanisms to address metal-induced stress. Ni(II) for example is sequestered by bonding with organic sulfur (R-SH) on the cysteine residues of peptides (Meagher 2000). Sequestration of Zn into shoot vacuoles has also been suggested to enhance tolerance in the Zn-hyperaccumulator plant *Thlaspi caerulescens* (Lasat et al. 2000). Several mechanisms have been proposed to account for Zn inactivation in vacuoles, including precipitation as Zn-phytate (Van Steveninck et al. 1990) and binding to low molecular weight organic acids (Salt 1999). Cadmium, a potentially toxic metal, also accumulates in plants, where it is detoxified by binding to phytochelatins (PC) (Vogeli-Lange and Wagner 1990; Salt and Rauser 1995), a family of thiol (SH)-rich peptides (Grill et al. 1987). Similarly, metallothionein (MT) compounds (proteins) have heavy metal-binding properties (Tomsett and Thurman 1988) and exist in numerous animals, and more recently, have been found in several plant and bacterial species (Kagi 1991). After the PC structures were elucidated, these peptides were found to be widely distributed in the plant kingdom, and were proposed to be functional equivalents of MTs (Grill et al. 1987). The role of PCs and MTs in metal detoxification has been well documented by Cobbett (2000). Apart from PCs, plants have also developed good tolerance mechanisms against the oxidative stress induced by heavy-metal exposure (Dat et al. 2000; Mishra et al. 2006).

Aerobic organisms are frequently exposed to ROS (reactive oxygen species). ROS is a generic term embracing not only free radicals, such as superoxide and hydroxyl radicals, but also H_2O_2 and singlet oxygen. These incompletely reduced oxygen species are toxic by-products, generated at low levels even in non-stressed plant cells within the chloroplasts and mitochondria during the redox reactions of photosynthetic electron transport and respiration. Stressful conditions generate more ROS; such stress may be from pathogen attacks, herbivore feeding, or exposures to UV light, heavy metals, or other substances (Diaz et al. 2001). Heavy-metal induced stress is often associated with degenerative reactions that are primarily associated with ROS; it is well known that their production in plant cells must be minimized to limit the effects of their high reactivity (Noctor and Foyer 1998). Toxicity generated by both O_2 and H_2O_2 is presumed to result from their ability to initiate reaction cascades that produce hydroxyl radicals capable of causing lipid peroxidation, protein denaturation, and DNA mutations. ROS may also damage the pigment system and the chlorophyll (Schutzendubel and Polle 2002; Sudo et al. 2008). Notwithstanding, nature has equipped plants with active antioxidant systems, which scavenge the toxicity generated by ROS (Cobbett 2000; Hou et al. 2007; Skorzynska-Polit et al. 2010). Such scavenging occurs by the synchronous action of several antioxidant enzymes that includes superoxidase dismutase (SOD), which converts superoxide to H_2O_2 (Bowler et al. 1992). Catalases (CATs) help convert H_2O_2 to water and molecular oxygen in peroxisomes (Noctor and Foyer 1998). In addition, an alternative mode of H_2O_2 destruction exists via the action of peroxidases (POD), which are found throughout the cell and have a much greater affinity for H_2O_2 than do CATs (Jimenez et al. 1997). Enzymes that exist in the ascorbate–glutathione cycle, where H_2O_2 is scavenged, are also highly active. In this cycle, the ascorbate peroxidases (APX) catalyze the reduction of H_2O_2 to water by ascorbate, and the resulting dehydroascorbate is reduced back to ascorbate with help from glutathione reductases (GRs) (Iturbe-Ormaetxe et al. 2001). Although plants have the ability to combat negative consequences of heavy-metal stress via the presence of antioxidants, many studies have shown that exposure to elevated concentrations of reactive metals reduce, rather than increase, antioxidative enzyme activities (Schutzendubel and Polle 2002). Because multiple factors affect heavy-metal tolerance in plants it is difficult to elucidate the exact tolerance mechanisms involved. However, two promising strategies are emerging to remediate polluted sites; the first is plant growth enhancement, and the second is reduced metal translocation, both of which are caused by soil amendments and/or microbial inoculations (PGPR) to multicontaminated sites.

3 Plant Growth-Promoting Bacteria

The beneficial free-living soil bacteria that exist in association with the roots of many different plants are generally referred to as plant growth-promoting rhizobacteria (PGPR) (Kloepper and Schroth 1978). Depending on their relationship with the host plants, PGPR can be divided into two major groups: (1) symbiotic rhizobacteria, which may invade the interior of cells and survive inside the cell (also called intracellular PGPR, e.g., nodule bacteria), and (2) free-living rhizobacteria that exist outside plant cells (called extracellular PGPR, e.g., *Bacillus*, *Pseudomonas*, *Burkholderia*, and *Azotobacter*) (Khan 2005; Babalola and Akindolire 2011). The major factor that affects the high concentration of bacteria found in the rhizosphere

Table 1 Plant growth-promoting rhizobacteria and the growth-regulating compounds associated with them

Organisms	Growth regulators	References
Pseudomonas chlororaphis, Arthrobacter pascens	Phosphate solubilization	Yu et al. (2012)
Pseudomonas spp.	Indole acetic acid (IAA), siderophore, P-solubilization	Li and Ramakrishna (2011)
Achromobacter xylosooxidans	IAA, P solubilisation	Ma et al. (2009)
Pseudomonas spp., *Bacillus megaterium*	IAA, siderophore and P solubilization	Rajkumar and Freitas (2008)
Microbacterium G16	IAA, siderophores	Sheng et al. (2008)
Azotobacter, *Fluorescent pseudomonas*, and *Bacillus*	IAA, siderophore, ammonia, HCN, P-solubilization	Ahmad et al. (2008)
Bacillus spp.	IAA, P solubilization, siderophores, HCN, ammonia	Wani et al. (2007)
Pseudomonas and *Bacillus*	Siderophores, IAA, P-solubilization	Rajkumar et al. (2006)
Brevibacillus brevis	IAA	Vivas et al. (2006)
Bravibacterium sp.	Siderophore	Noordman et al. (2006)
Xanthomonas sp. *RJ3*, *Azomonas* sp. *RJ4*, *Pseudomonas* sp. *RJ10*, *Bacillus* sp. *RJ31*	IAA	Sheng and Xia (2006)
Bacillus subtilis	IAA and P-solubilization	Zaidi et al. (2006)
Bacillus sp.	P-solubilization	Canbolat et al. (2006)
Variovorax paradoxus, *Rhodococcus* sp. and *Flavobacterium* (Cd tolerant)	IAA and siderophores	Belimov et al. (2005)
Sphingomonas sp., *Mycobacterium* sp., *Bacillus* sp., *Rhodococcus* sp*., Cellulomonas* sp. and *Pseudomonas* sp.	IAA	Tsavkelova et al. (2005)
Micrococcus luteus	IAA, P-solubilization	Antoun et al. (2004)
Bacillus, *Pseudomons*, *Azotobacter*, and *Azospirillum*	P-solubilization and IAA	Tank and Saraf (2003)
Pseudomonas fluorescence	Siderophore	Khan et al. (2002)
Kluyvera ascorbata	Siderophore	Burd et al. (2000)

is the presence of high nutrient levels (especially small molecules such as amino acids, sugars, and organic acids) that are exuded from the roots of most plants (Bayliss et al. 1997; Penrose and Glick 2001). PGPR can positively influence plant growth and development in three different ways, in that they

1. Synthesize and provide growth-promoting compounds to the plants (Glick 1995) (Table 1),
2. Facilitate the uptake of certain environmental nutrients such as nitrogen, phosphorus, sulfur, magnesium, and calcium (Bashan and Levanony 1990; Belimov and Dietz 2000; Cakmakci et al. 2006), and
3. Decrease or prevent some deleterious effects caused by phytopathogenic organisms or other diseases (Khan et al. 2002; Lugtenberg and Kamilova 2009)

Generally, rhizobacteria improve plant growth by synthesizing phytohormone precursors (Ahmad et al. 2008), vitamins, enzymes, siderophores, and antibiotics (Burd et al. 2000; Noordman et al. 2006). PGPR also increase plant growth by synthesizing specific enzymes, which induce biochemical changes in plants. For example, ethylene plays a critical role in various plant developmental processes, such as leaf senescence and abscission, epinasty, and fruit ripening (Vogel et al. 1998). Ethylene also regulates node factor signaling, nodule formation, and has primary functions in plant defense systems. Moreover, as a result of the plant infection by rhizobacteria, ethylene production is increased (Boller 1991), which, at higher concentrations, will inhibit plant growth and development (Morgan and Drew 1997; Grichko and Glick 2001). However, bacterial 1-aminocyclopropane-1-carboxylate (ACC), a deaminase synthesized by PGPR (Babalola et al. 2003; Madhaiyan et al. 2006; Rajkumar et al. 2006), alleviates stress induced by such ethylene-mediated impact. In addition, rhizobacterial strains can solubilize inorganic P (Glick et al. 1998; Yu et al. 2012), or mineralize organic P, and thereby improve plant stress tolerance to drought, salinity, and metal toxicity (Ponmurugan 2006; Khan et al. 2007). Pishchik et al. (2005) mathematically simulated the succession of events that began with phytohormone (IAA and ethylene) synthesis and ended with higher uptake of ions by roots, under conditions of cadmium stress. Possibly, synthesis of phytohormones might be stimulated by exposure to heavy metals. Conversely, these processes may be hindered by high heavy-metal concentrations (DellAmico et al. 2005), because many rhizobacteria cannot survive when such concentrations are high. These authors further reported that many different microbial communities are able to withstand high heavy-metal concentrations when living in association with rhizospheric soils and the rhizoplane.

The mechanism of metal tolerance and the possible metal transforming capacities of the metal-resistant PGPR are briefly discussed in the following section.

3.1 How Do PGPR Combat Heavy-Metal Stress?

Unlike many pollutants, which undergo biodegradation to produce less toxic, less mobile, and less bioavailable products, removing heavy metals from a contaminated environment is much more difficult. Heavy metals cannot be degraded biologically and are ultimately indestructible, though the speciation and bioavailability of metals may change as environmental factors change. Some metals (e.g., zinc, copper, nickel, and chromium) are essential and beneficial micronutrients for plants, animals, and microorganisms (Olson et al. 2001), whereas others (e.g., cadmium, mercury, and lead) have no known biological or physiological function (Gadd 1992). However, high concentrations of these heavy metals greatly affect microbial communities, and may reduce their total microbial biomass (Giller et al. 1998), their activity (Romkens et al. 2002), or change microbial community structure (Gray and Smith 2005). Therefore, at higher concentrations, either heavy-metal ions completely inhibit a microbial population by inhibiting its various metabolic activities or these organisms may develop resistance or tolerance to such elevated levels of heavy metals. This ability to live and grow under in the presence of high metal concentration exists in many rhizospheric microorganisms. Ledin (2000) explained the difference between microbial tolerance and resistance; he defines tolerance as the ability to cope with metal toxicity by means of intrinsic properties of the microorganisms, whereas resistance is the ability of microbes to detoxify heavy metals by being activated in direct response to the high heavy-metal concentrations.

Toxic heavy-metal pollutants should be either completely removed from the contaminated soil or transformed or immobilized in ways that render them safe. For survival under metal-stressed environment, PGPR have developed a range of mechanisms by which they can immobilize, mobilize, or transform heavy metals, thereby rendering them inactive (Nies 1999). These mechanisms include (1) exclusion-metal ions that are kept away from target sites, (2) extrusion-metals that are pushed out of the cell through chromosomal/plasmid mediated events (3) accommodation—metals that form complexes with metal-binding proteins, e.g., mettalothioneins, low molecular weight proteins (Kao et al. 2006; Umrania 2006), and other cell components, (4) biotransformation, in which the toxic metal is reduced to less toxic forms, and (5) methylation and demethylation. One or more of the above-mentioned mechanisms allow the microbes to function metabolically in metal-contaminated sites/soils. Interest in exploiting these bacterial properties to remediate heavy-metal contaminated sites is growing, and early results from their application are promising (Lloyd and Lovley 2001; Hallberg and Johnson 2005).

3.2 Synergistic Interaction of PGPR and Plants in Heavy-Metal Remediation

Although many plant–microbe interactions have been investigated, the studies performed so far have mainly emphasized plant–pathogen interactions. Only 10 years ago, research on the ecology of microbes in the rhizosphere was focused on the microbiological detoxification and decontamination of soil as affected by heavy metals. The fact that PGPR promotes plant growth is well documented (Reed and Glick 2004; Babalola et al. 2007; Babalola 2010), and more recently, PGPR have been successfully used to reduce plant stress in metal-contaminated soils. The microorganisms that are associated with roots establish a synergistic relationship with plant roots which enhances nutrient absorption and improves plant performance, as well as the quality of soils (Tinker 1984; Yang et al. 2009). Bacteria interact with and affect plant growth in a variety of ways. Some bacteria are phytopathogenic and actively inhibit plant growth; others (e.g., PGPR) facilitate plant growth through several mechanisms; many soil bacteria do not appear to affect the plant growth at all, although a change in soil conditions could reverse this (Glick 1995). Some microbial communities have the ability to sequester heavy metals, and therefore may be useful for bioremediating contaminated areas (Hallberg and Johnson 2005; Umrania 2006). When microbes are used to bioremediate a contaminated site, plant-associated bacteria can be potentially used to improve phytoextraction activities by altering the solubility, availability, and transport of heavy metals, and nutrients as well, by reducing soil pH and releasing chelators (Ma et al. 2011). Among the metabolites produced by PGPR, siderophores play a significant role in metal mobilization and accumulation (Rajkumar et al. 2010). Recently, Cr and Pb were found to be released into the soil solution after soil was inoculated with *P. aeruginosa* (Braud et al. 2009). *P. aeruginosa* can realistically only serve as a model system, because it is a well-known pathogen, and regulators would not allow deliberate release of it to the environment. Although no field success has yet been achieved by doing so, the concept of inoculating seeds/rhizospheric soils with selected metal-mobilizing bacteria to improve phytoextraction in metal-contaminated soils has merit.

3.3 ACC Deaminase and Plant Stress Reduction from Ethylene

As mentioned earlier, ethylene is produced under normal plant-growth conditions and regulates plant growth, although it is toxic to plants at higher concentrations (Bestwick and Ferro 1998). Ethylene is produced from L-methionine via the intermediates *S*-adenosyl-L-methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) (Yang and Hoffman 1984). In a step-wise metabolic reaction, methionine is first converted into *S*-adenosyl-L-methionine by SAM synthetase (Giovanelli et al. 1980) and SAM is then hydrolyzed to ACC and 5-methyl thioadenosine (Kende 1989) by ACC synthetase. Finally, ACC is metabolized to ethylene, CO_2, and cyanide by ACC oxidase (John 1991). Several chemicals have been used to control ethylene-mediated stress in plants. Unfortunately, use of such substances is environmentally unfriendly. Applying cyclopropenes can block the action of ethylene and it can potentially be used to extend the shelf life of flowers, and potted plants. Other compounds are known to inhibit ethylene biosynthesis, although they are potentially harmful to the environment, e.g., silver thiosulfate (Bestwick and Ferro 1998).

Generally, it is important that ethylene levels in plants be kept at minimum levels possible. This can be accomplished by minimizing the ethylene precursor ACC, which is subject to degradation by an ACC enzyme isolated from a *Pseudomonas* sp. strain ACP and from yeast *Hansenula saturnus* (Honma and Shimomura 1978; Minami et al. 1998). An ACC deaminase has also been detected in the fungus *Penicillium citrinum*, and bacterial strains originating from the soil that have ACC deaminase activity have been reported (Glick 1995; Jia et al. 2000; Belimov et al. 2001; Babalola et al. 2003). This enzyme, ACC deaminase, degrades ACC to ammonia and α-ketobutyrate, and can be utilized to protect plants from ethylene-generated stress (Glick et al. 1998). Many plant species require ethylene for seed germination, and ethylene production increases during seed germination and seedling growth (Abeles et al. 1992). However, elevated ethylene levels may inhibit root elongation and depress growth (Morgan and Drew 1997). In higher plants,

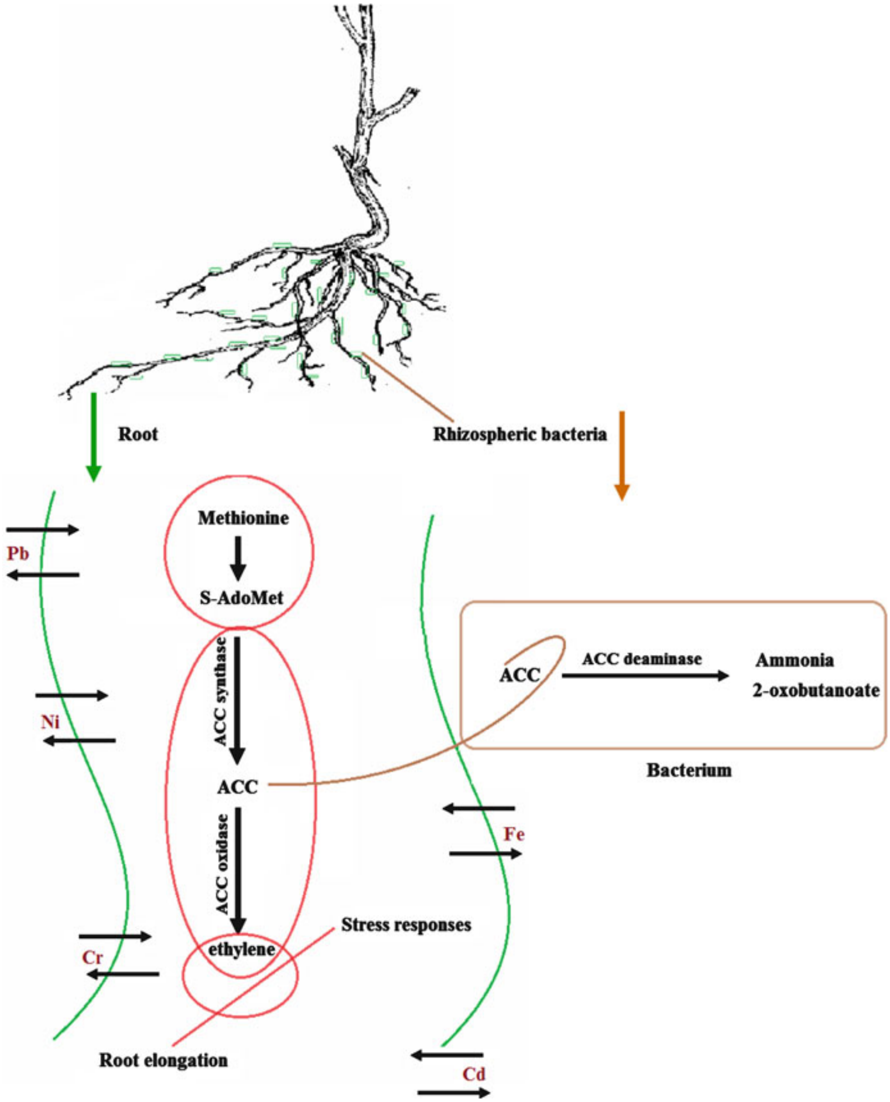

Fig. 1 Diagrammatic model showing the process for reducing ethylene levels in roots by using bacterial 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. ACC synthesized in plant tissues is believed to be exuded from plant roots and is taken up by rhizobacteria where ACC is hydrolyzed to ammonia and 2-oxobutanoate

S-Adenosyl-L-methionine (S-AdoMet) is synthesized from methionine and ACC is synthesized and converted into ethylene by ACC oxidase. Further ethylene production in plants is controlled by regulating the expression of ACC synthase and ACC oxidase genes (Kim et al. 2001). In Fig. 1, a model of the process by which ethylene levels are reduced in roots is presented, along with the role played by the ACC enzyme (Glick et al. 2007).

PGPR synthesize the indole-3-acetic acid (IAA) utilizing tryptophan excreted by roots in the rhizospheric region. The synthesized IAA molecules are then secreted and transported into plant cells. These auxins have dual roles. One is to participate in plant cell growth and the other is to promote ACC synthase activity to increase the ethylene titer. Stress induces an increase in ACC levels and, therefore, emulates the action of IAA molecules. Increased ACC molecules then diffuse from plants and are imported into PGPR cells where they are subjected to the action of ACC deaminase. Because of this, microbes and plants are more tolerant to stress-induced growth inhibition that is mediated by ethylene. When tested, strains of ACC deaminase-containing plant growth-promoting bacteria were found to reduce the amount of ACC that was detectable by HPLC, and the ethylene levels in canola seedlings were also lowered (Penrose and Glick 2001). Thus PGPR can potentially be used to counter ethylene-mediated stress, although field trials are needed to elucidate the mechanism by which this occurs.

4 Summary

In this review, we briefly describe the biological application of PGPR for purposes of phytoremediating heavy metals. We address the agronomic practices that can be used to maximize the remediation potential of plants. Plant roots have limited ability to absorb metals from soil, mainly because metals have low solubility in the soil solution. The phytoavailability of metal is closely tied to the soil properties and the metabolites that are released by PGPR (e.g., siderophores, organic acids, and plant growth regulators). The role played by PGPR may be accomplished by their direct effect on plant growth dynamics, or indirectly by acidification, chelation, precipitation, or immobilization of heavy metals in the rhizosphere.

From performing this review we have formed the following conclusions:

- The most critical factor in determining how efficient phytoremediation of metal-contaminated soil will be is the rate of uptake of the metal by plants. In turn, this depends on the rate of bioavailability. We know from our review that beneficial bacteria exist that can alter metal bioavailability of plants. Using these beneficial bacteria improves the performance of phytoremediation of the metal-contaminated sites.
- Contaminated sites are often nutrient poor. Such soils can be nutrient enriched by applying metal-tolerant microbes that provide key needed plant nutrients. Applying metal-tolerant microbes therefore may be vital in enhancing the detoxification of heavy-metal-contaminated soils (Glick 2003).
- Plant stress generated by metal-contaminated soils can be countered by enhancing plant defense responses. Responses can be enhanced by alleviating the stress-mediated impact on plants by enzymatic hydrolysis of ACC, which is intermediate in the biosynthetic pathway of ethylene. These plant–microbe partnerships can act as decontaminators by improving phytoremediation.

Soil microorganisms play a central role in maintaining soil structure, fertility, and in remediating contaminated soils. Although not yet widely applied, utilizing a plant–microbe partnership is now being recognized as an important tool to enhance successful phytoremediaton of metal-contaminated sites. Hence, soil microbes are essential to soil health and sustainability. The key to their usefulness is their close association with, and positive influence on, plant growth and function. To capitalize on the early success of this technique and to improve it, additional research is needed on successful colonization and survival of inoculums under field conditions, because these are vital for the success of this approach. In addition, the effects of the interaction of PGPR and plant root-mediated process on the metal mobilization in soil are required, to better elucidate the mechanism that underlies bacterial-assisted phytoremediation. Finally, applying PGPR-associated phytoremediation under field conditions is important, because, to date, only locally contaminated sites have been treated with this technique, by using microbes cultured in the laboratory.

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