Role of Rhizobacteria in phytoremediation of heavy metals: An overview

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Heavy metal pollution of soil is a significant environmental problem and has its negative impact on human health and agriculture. Rhizosphere, as an important interface of soil and plant, plays a significant role in phytoremediation of contaminated soil by heavy metals, in which, microbial populations are known to affect heavy metal mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization and redox changes, and therefore, have potential to enhance phytoremediation processes. Phytoremediation strategies with appropriate heavy metal-adapted rhizobacteria have received more and more attention. A heavy metal-resistant bacterial strain was isolated from heavy metal-contaminated soils and identified as *Burkholderia* sp. J62 based on the 16S rDNA gene sequence analysis. Strain J62 was able to colonize and develop in the rhizosphere soil of maize and tomato after root inoculation and was able to promote the growth of maize and tomato. The application of strain J62 effectively increased the bioavailability of Pb and Cd in the rhizosphere soils and promoted the growth of maize and tomato plants, consequently increasing the total Pb and Cd uptakes of the plants even under nonsterile conditions. The isolate was found to exhibit different multiple heavy metal and antibiotic resistance characteristics. Inoculation with the isolate was found to increase the biomass of maize and tomato plants. Increase in tissue Pb and Cd contents varied from 38 to 192% and from 5 to 191% in inoculated plants growing in heavy metal-contaminated soils compared to the uninoculated control, respectively. Phytoremediation could clean up the heavy metal-contaminated soil. In addition, although strain J62 significantly increased the Pb and Cd uptakes by the maize and tomato, the total Pb and Cd accumulation of the plants is low.

Keywords: Rhizobacteria, *burkholderia* sp., phytoremediation, heavy metals, rhizosphere, solubilization.

INTRODUCTION

Heavy metals are conventionally defined as elements with metallic properties (ductility, conductivity, stability as cations, ligand specificity, etc.) and an atomic number >20. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb and Ni. Metals are natural components in soil with a number of heavy metals being required by plants as micronutrients. "Heavy metals" are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4°C (39°F). Simply stated, specific gravity is a measure of density of a given amount of a solid substance when it is compared to an equal amount of water. Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic, 5.7; cadmium, 8.65; iron, 7.9; lead, 11.34; and mercury, 13.546 (Lide, 1992). There are 35 metals that concern us because of occupational or residential exposure; 23 of these are the heavy elements or "heavy metals": antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc. Interestingly, small amounts of these elements are common in our environment and diet and are actually necessary for good health, but large amounts of any of them cause acute or
chronic toxicity (poisoning) (Glanze, 1996; Chabot, et al., 1996). For some heavy metals, toxic levels can be just above the background concentrations naturally found in nature. Therefore, it is important for us to inform ourselves about the heavy metals and to take protective measures against excessive exposure. In most parts of the United States, heavy metal toxicity is an uncommon medical condition; however, it is a clinically significant condition when it does occur. If unrecognized or inappropriately treated, toxicity can result in significant illness and reduced quality of life (Ferner, 2001). For persons who suspect that they or someone in their household might have heavy metal toxicity, testing is essential. Appropriate conventional and natural medical procedures may need to be pursued (Dupler, 2001).

Pollution of soils with heavy metals is becoming one of the most severe environmental and human health hazards. Elevated levels of heavy metals not only decrease soil microbial activity and crop production, but also threaten human health through the food chain (Mclaughlin et al., 1999). Excessive accumulation of heavy metals is toxic to most plants. Heavy metals ions, when present at an elevated level in the environment, are excessively absorbed by roots and translocated to shoot, leading to impaired metabolism and reduced growth (Bingham et al., 1986; Foy et al., 1978). Heavy metal contamination to water and soil poses a major environmental and human health problem. In addition, excessive metal concentrations in contaminated soils result in decreased soil microbial activity and soil fertility, and yield losses (McGrath et al., 1995). Cadmium (Cd), as a non-essential, toxic heavy metal to plants, which may well demonstrate the problem, can inhibit root and shoot growth, affect nutrient uptake and homeostasis, and is frequently accumulated by agriculturally important crops (Sanità di Toppi and Gabrielli; 1999 and Yan et al., 2007). Thus, when Cd-enriched crop products are consumed by animals and humans, it can cause diseases. On condition that soil Cd pollution is cumulative with levels increasing over time, the soil may eventually become unusable for crop production. Similarly, contamination of soil with Cd can negatively affect biodiversity and the activity of soil microbial communities (McGrath, 1994). Burd et al. (1998)’s experiments revealed that canola seeds developed normally in the presence of up to 1 mmol/L nickel chloride, but that plant root and shoot elongation were inhibited at higher levels. Phytoextraction is emerging as a potential cost effective solution for the remediation of heavy metal-contaminated soils in opposition to the conventional chemical and physical remediation technologies that are generally too costly and often harmful to soil characteristics (Blaylock et al., 1997 and Quartacci et al., 2006). In recent years, some plant species (identified as hyperaccumulators) growing in heavy metal-contaminated sites have been found with the ability to accumulate unusually high concentrations of heavy metals without impacting on their growth and development (Baker and Brooks, 1989). However, most hyperaccumulators identified so far are not suitable for field phytoremediation applications due to their small biomass and slow growth (Shen and Liu, 1998). In addition, a large proportion of many metals is adsorbed or occluded by carbonates, organic matters, Fe–Mn oxides and primary or secondary minerals (Garbisu and Alkorta, 2001). Low bioavailability of heavy metals in soils may also limit the efficiency of phytoremediation (Kayser et al., 2000, Chen et al., 2004 and Sheng and Xia, 2006).

Soil microorganisms can affect trace metal mobility and availability to the plants (Abou-Shanab et al., 2003 and Idris et al., 2004). For example, the presence of rhizosphere (defined as the volume of soil adjacent to and influenced by the plant root) (Smalla et al., 2001) bacteria increased the uptake of Cd in Brassica napus (Sheng and Xia, 2006) and Ni in Alyssum murale (Abou-Shanab et al., 2006). Arbuscular mycorrhizal (AM) fungi are ubiquitous symbiotic associations found in both natural and heavy metal-contaminated sites (Wang et al., 2007). AM fungi may stimulate phytorextraction by essentially improving plant growth and increasing the total metal uptake (Wang et al., 2007). Mycorrhizal fungi associated with these plants play a great role in the establishment of these plants on the contaminated soils. Therefore, the application of heavy metal-solubilizing microorganisms is a promising approach for increasing heavy metal bioavailability in heavy metal amended soils. In addition, bacteria producing indole acetic acid, siderophores and 1-aminocyclopropane-1-carboxylate deaminase and phosphate-solubilizing bacteria are capable of stimulating plant growth (Glick et al., 1995, Chabot et al., 1996 and Rajkumar et al., 2006). Plant growth promoting bacteria (PGPB) could improve plant competitiveness and responses to external stress factors (Egamberdiyeva and Hflch, 2004). However, little is known about the potential of heavy metal-resistant and heavy metal-solubilizing bacteria on the phytorextraction of Pb and Cd from heavy metal-contaminated soils. The objectives of this study were to isolate and characterize heavy metal-resistant and heavy metal-solubilizing bacteria from heavy metal-contaminated soils, and to select PGPB strains which might be useful to increase plant biomass production and Pb and Cd uptakes by plants under unfavorable environmental conditions for improving the efficiency of phytorextraction of Pb- and Cd-polluted soils. However, pollution of biosphere by toxic metals has accelerated dramatically since the beginning of the industrial revolution. As a result of human activities such as mining and smelting of metals, electroplating, gas exhaust, energy and fuel production, fertilizer, sewage and pesticide application, municipal waste generation, etc. (Kabata-Pendias and Pendias, 1989), metal pollution has become one of the most severe environmental problems today. Phytoremediation holds promise for in situ treatment of...
heavy metal contaminated soils. Recently, the benefits of combining siderophore-producing bacteria (SPB) with plants for metal removal from contaminated soils have been demonstrated. Metal-resistant SPB plays an important role in the successful survival and growth of plants in contaminated soils by alleviating the metal toxicity and supplying the plant with nutrients, particularly iron. Furthermore, bacterial siderophores are able to bind metals other than iron and thus enhance their bioavailability in the rhizosphere of plants. Overall, an increase in plant growth and metal uptake will further enhance the effectiveness of phytoremediation processes (Rajkumar, 2010). The present review study indicated the role of bacterial activities in the photoremediation of heavy metals from the soil in order to prevent the environmental pollution.

Remediation technologies

Heavy metals cannot be destroyed biologically (no "degradation", change in the nuclear structure of the element, occurs) but are only transformed from one oxidation state or organic complex to another (Garbisu and Alkorta, 2001), remediation of heavy metal contamination in soils is more difficult. Until now, methods used for their remediation such as excavation and land fill, thermal treatment, acid leaching and electroreclamation are not suitable for practical applications, because of their high cost, low efficiency, large destruction of soil structure and fertility and high dependence on the contaminants of concern, soil properties, site conditions, and so on. Thus, the development of phytoremediation strategies for heavy metals contaminated soils is necessary (Chaney et al., 2000; Cheng et al., 2002; Lasat, 2002).

The roots of plants interact with a large number of different microorganisms, with these interactions being major determinants of the extent of phytoremediation (Glick, 1995). The functioning of associative plant-bacterial symbioses in heavy-metal-polluted soil can be affected from the side of both the micropartner (plant-associated bacteria) and the host plant. Soil microbes play significant roles in recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals, and control of plant pests and plant growth (Elsgaard et al., 2001; Filip, 2002; Giller et al., 1998). Thus, bacteria can augment the remediation capacity of plants or reduce the phytotoxicity of the contaminated soil. In addition, plants and bacteria can form specific associations in which the plant provides the bacteria with a specific carbon source that induces the bacteria to reduce the phytotoxicity of the contaminated soil. Alternatively, plants and bacteria can form nonspecific associations in which normal plant processes stimulate the microbial community, which in the course of normal metabolic activity degrades contaminants in soil. Plants roots can provide root exudate, as well as increase ion solubility. These biochemical mechanisms increase the remediation activity of bacteria associated with plant roots. To sum up, the adaptation capabilities of both partners of the associative symbiosis as well as the bioremediation potential of the microsymbiont are of importance in minimizing the detrimental effect of heavy-metal pollution.

Technogenic activities (industrial-plastic, textiles, microelectronics, wood preservatives; mining-mine refuse, tailings, smelting; agrochemicals-chemical fertilizers, farm yard manure, pesticides; aerosols-pyrometallurgical and automobile exhausts; biosolids-sewage sludge, domestic waste; fly ash-coal combustion products) are the primary sources of heavy metal contamination and pollution in the environment in addition to geogenic sources. During the last two decades, bioremediation has emerged as a potential tool to clean up the metal-contaminated/polluted environment. Exclusively derived processes by plants alone (phytoremediation) are time-consuming. Further, high levels of pollutants pose toxicity to the remediating plants. This situation could be ameliorated and accelerated by exploring the partnership of plant-microbe, which would improve the plant growth by facilitating the sequestration of toxic heavy metals. Plants can bioconcentrate (phytoextraction) as well as bioimmobilize or inactivate (phytostabilization) toxic heavy metals through in situ rhizospheric processes. The mobility and bioavailability of heavy metal in the soil, particularly at the rhizosphere where root uptake or exclusion takes place, are critical factors that affect phytoextraction and phytostabilization. Developing new methods for either enhancing (phytoextraction) or reducing the bioavailability of metal contaminants in the rhizosphere (phytostabilization) as well as improving plant establishment, growth, and health could significantly speed up the process of bioremediation techniques (Ma et al., 2011).

Heavy metal-bacteria interactions

Rhizobacteria have been shown to possess several traits that can alter heavy metals bioavailability (Lasat, 2002; McGrath et al., 2001; Whiting et al., 2001) through the release of chelating substances, acidification of the microenvironment, and by influencing changes in redox potential (Smith and Read, 1997). For example, Abou-Shanab et al. (2003a) reported that the addition of Sphingomonas macrogoltabidus, Microbacterium liquefaciens, and Microbacterium arabinogalactanolyticum to Alyssum murale grown in serpentine soil significantly increased the plant uptake of Ni when compared with the un-inoculated controls as a result of soil pH reduction. However, heavy metals are known to be toxic to plants and most organisms when present in soils in excessive concentrations. Giller et al.
(1998) reported that there was a detrimental effect to soil microbial diversity and microbial activities (indexes of microbial metabolism and of soil fertility) in metal-polluted environments. Most of the commonly known heavy metal accumulators belong to the Brassicaceae family (Kumar et al., 1995). Although hyperaccumulator plants have exceptionally high metal accumulating capacity, most of these have a slow growth rate and often produce limited amounts of biomass when the concentration of available metal in the contaminated soil is very high. An alternative is to use species with a lower metal accumulating capacity but higher growth rates, such as Indian mustard (Brassica juncea); another alternative is to provide them with an associated plant growth-promoting rhizobacteria, which also is considered to be an important component of phytoremediation technology (Wenzel et al., 1999; Glick, 2003). Obviously, the rhizosphere contains a large microbial population with high metabolic activity compared to bulk soil (Anderson et al., 1993). Microbial populations are known to affect heavy metals mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization, and redox changes (Abou-Shanab et al., 2003a; Smith and Read, 1997). Especially, some plant growth-promoting bacteria associated with plant roots also may exert some beneficial effects on plant growth and nutrition through a number of mechanisms such as N₂ fixation, production of phytohormones and siderophores, and transformation of nutrient elements when they are either applied to seeds or incorporated into the soil (Kloeper et al., 1989; Glick, 1995; Glick et al., 1999). The use of rhizobacteria in combination with plants is expected to provide high efficiency for phyto remediation (Abou-Shanab et al., 2003a; Whiting et al., 2001). Therefore, the potential and the exact mechanism of rhizobacteria to enhance phyto remediation of soil heavy metals pollution have recently received some attention (de Souza et al., 1999a; Whiting et al., 2001). For example, Burd et al.(1998) observed that both the number of Indian mustard seeds that germinated in a nickel-contaminated soil, and the attainable plant size increased by 50–100% by the addition of K. ascorbata SUD165/26, an associated plant growth-promoting rhizobacteria, to the soil in preliminary field trials, and de Souza et al.(1999b) investigated phyto remediation of Se and Hg in constructed wetlands and found that accumulation of Se and Hg were enhanced by rhizobacteria in wetland plant tissues.

**Plant-bacteria-soil interactions**

The specificity of the plant-bacteria interaction is dependent upon soil conditions, which can alter contaminant bioavailability, root exudate composition, and nutrient levels. In addition, the metabolic requirements for heavy metals remediation may also dictate the form of the plant-bacteria interaction i.e. specific or nonspecific. Along with metal toxicity, there are often additional factors that limit plant growth in contaminated soils including arid conditions, lack of soil structure, low water supply and nutrient deficiency.

Rhizosphere microorganisms, which are closely associated with roots, have been termed plant growth promoting rhizobacteria (PGPR) (Glick, 1995). Plant growth-promoting rhizobacteria include a diverse group of free-living soil bacteria that can improve host plant growth and development in heavy metal contaminated soils by mitigating toxic effects of heavy metals on the plants (Belimov et al., 2004). It is well known that heavy metals can even be toxic for metal-accumulating and metal-tolerant plants, if the concentration of metals in the environment is sufficiently high. This is partly attributable to iron deficiency in a range of different plant species (Mishra and Kar, 1974; Ma and Nomoto, 1993; Römheld and Marschner, 1986; Wallace et al., 1992) in heavy metal contamination soil. Furthermore, the low iron content of plants that are grown in the presence of high levels of heavy metals generally results in these plants becoming chlorotic, since iron deficiency inhibits both chloroplast development and chlorophyll biosynthesis (Imsande, 1998). However, microbial iron-siderophore complexes can be taken up by plants, and thereby serve as an iron source for plants (Bar-Ness et al., 1991; Reid et al., 1986; Wang et al., 1993). It was therefore reasoned that the best way to prevent plants from becoming chlorotic in the presence of high levels of heavy metals was to provide them with an associated siderophore-producing bacterium. This suggests that some plant growth-promoting bacteria can significantly increase the growth of plants in the presence of heavy metals including nickel, lead, and zinc (Burd et al., 1998; 2000), thus allowing plants to develop longer roots and get better established during early stages of growth (Glick et al., 1998). Once the seedling is established, the bacterium can also help the plant acquire sufficient iron for optimal plant growth. Similarly, chromium-resistant pseudomonads, isolated from paint industry effluents, were able to stimulate seed germination and growth of Triticum aestivus in the presence of potassium dichromate (Hasnain and Sabri, 1996). In this case, the bacterial enhancement of seedling growth was associated with reduced chromium uptake. The effect of adding K. ascorbata SUD165, a plant growth-promoting bacterium, to canola or tomato seeds before the seeds germinate, was also examined in the presence of inhibitory concentrations of Ni²⁺. The results of these experiments showed that at all concentrations of nickel tested (1 to 6 mmol/L Ni²⁺), using both a low-level and a high-level bacterial cell treatment (cell suspension absorbance of 0.025 or 0.50, respectively), with both canola and tomato plants, roots and shoots, and in both pouches and pots, the addition of K. ascorbata SUD165 significantly decreased the toxicity of the added nickel (Burd et al., 1998).
Bacteria in the rhizosphere are involved in the accumulation of potentially toxic trace elements into plant tissues. De Souza et al. (1999b) found that axenic saltmarsh bulrush plants supplied with different rhizosphere bacteria accumulated (70±80) % higher Se concentrations in their roots and (40±60) % higher Se concentrations in shoots than plants grown under axenic conditions. Four out of the six bacterial strains tested significantly enhanced Se accumulation in roots and shoots of axenic plants when they were added as pure cultures (P<0.05; n=3). A mixture of the six bacterial strains tested also enhanced Se accumulation in axenic bulrush plants. However, there were some opposite viewpoints that the presence of ectomycorrhizal or vesicular-arbuscular fungi on the roots of plants decreased the uptake of metals by the plants and thereby increased plant biomass (Bradley et al., 1982; Brown and Wilkins, 1985; Dueck et al., 1986; Heggo et al., 1990; Killham and Firestone, 1983; Tam, 1995). The reason might be that some plants involve the use of plant growth-promoting bacteria or mycorrhizal fungi to lessen the deleterious effects of heavy metals. Plant growth-promoting rhizobacteria (PGPR) are bacteria capable of promoting plant growth by colonizing the plant root. For a long period PGPR were mainly used for assisting plants to uptake nutrients from the environment or preventing plant diseases. Phytoremediation is a new and promising approach to remove contaminants in the environment. But using plants alone for remediation confronts many limitations. Recently, the application of PGPR has been extended to remediate contaminated soils in association with plants. Of all the present contaminants, the profound impacts of organic and heavy metal pollutants have attracted worldwide attention (Zhuang et al., 2007).

Phytoremediation assisted by soil rhizobacteria

Phytoremediation, the use of plants to extract, sequester, and/or detoxify pollutants through physical, chemical, and biological processes (Cunningham and Ow, 1996; Saxena et al., 1999; Wenzel et al., 1999), has been reported to be an effective, in situ, non-intrusive, low-cost, aesthetically pleasing, ecologically benign, socially accepted technology to remediate polluted soils (Alkorta and Garbisu, 2001; Garbisu et al., 2002; Weber et al., 2001). It also helps prevent landscape destruction and enhances activity and diversity of soil microorganisms to maintain healthy ecosystems, which is consequently considered to be a more attractive alternative than traditional methods to the approaches that are currently in use for dealing with heavy metal contamination (Bogardt and Hemmingsen, 1992; Cunningham and Berti, 1993; Cunningham and Ow, 1996; Cunningham et al., 1995; Salt et al., 1995). Phytoremediation of heavy metals may take one of several forms: phytoextraction, rhizofiltration, phytostabilization, and phytovolatilization.

Phytoextraction refers to processes in which plants are used to concentrate metals from the soil into the roots and shoots of the plant; rhizofiltration is the use of plant roots to absorb, concentrate or precipitate metals from effluents; and phytostabilization is the use of plants to reduce the mobility of heavy metals through absorption and precipitation by plants, thus reducing their bioavailability; phytovolatilization is the uptake and release into the atmosphere of volatile materials such as mercury- or arsenic-containing compounds. The ideal plant for phytoextraction should grow rapidly, produce a high amount of biomass, and be able to tolerate and accumulate high concentrations of metals in shoots.

Rhizospheric microorganism

The rhizobacteria of metal accumulating and hyper accumulating plants and their role in the tolerance to and uptake of heavy metals by the plants have been studied. Research has also shown that many rhizobacteria are tolerant to heavy metals and play important roles in mobilization or immobilization of heavy metals (Gadd, 1990). It is now well-clarified that the population of rhizobacteria is several orders of magnitude greater than that in the bulk soil with the elevated levels of heavy metals in these soils have significant impacts on microorganism population size, community structure, and overall activity of the soil microbial communities. Experiments showed that the number of bacteria in the rhizosphere of D. fusca reached 1.0×107 CFU/g. This relatively low bacterial count can be attributed to the presence of heavy metals in high concentrations (39 mg Co/kg, 3 mg Cd/kg, 79 mg Ni/kg, 30 mg Cu/kg, 4834 mg Zn/kg, 123 mg Cr/kg and 114 mg Pb/kg dry soil) (Abou-Shanab et al., 2005). Chaudri et al. (1992) also found that rhizobium populations were reduced at concentrations >7 mg/kg soil in their Cd treatments. Field studies of metal contaminated soils have similarly demonstrated that elevated metal loadings can result in decreased microbial community size (Brookes and McGrath, 1984; Chander and Brookes, 1991; Jordan and LeChevalier, 1975; Konopka et al., 1999). Besides, the microorganism community structure of the rhizosphere population is important in the context of plant growth. This is largely attributed to the finding that microbial populations often establish some sort of positive cooperation with the host plant system. For example, soil pollution with heavy metals could lead to the appearance of heavy-metal resistant rhizobacteria in the soil of industrial regions (Aleem et al., 2003). It was revealed that a high proportion of metal resistant bacteria persists in the rhizosphere of the hyperaccumulators Thalaspi caerulescens (Delorme et al., 2001) and Alyssum bertoloni (Mengoni et al., 2001) or Alyssum murale (Abou-Shanab et al., 2003a) grown in soil contaminated with Zn and Ni or Ni, respectively. The presence of rhizobacteria increased concentrations of Zn (Whiting et
al., 2001), Ni (Abou-Shanab et al., 2003b) and Se (de Souza et al., 1999a) in T. caerulescens, A. murale and B. juncea, respectively. Multiple metal-resistances (MMR) in bacteria seems to be the rule rather than the exception. Abou-Shanab et al., (2005) tested the patterns of tolerance of the heavy metals in the 107 rhizobacterial isolates at 1 mmol/L concentrations and found all the rhizobacterial strains to be tolerant to multiple metal ions. Strains with hexa-, penta-, tetra-, and tri-metal ions tolerant, respectively, were found more frequent than those with hepta-, double and mono-tolerance. Notably cadmium, copper, lead, and nickel resistance seemed to be restricted to those strains which were resistant to six metals or more. Similar observations have been previously reported by Sabry et al., (1997).

High levels of heavy metals could decrease rhizobacteria metabolic activity, biomass and diversity (Gremion et al., 2004; Sandaa et al., 1999). The activities of the large population of bacteria inhabiting the rhizosphere can also be expected to influence heavy metals uptake by plants. It is reported that under non-sterile soil system, plants showed no iron-deficiency symptoms and have fairly high iron level in roots in contrast to plants grown in sterile system. This can attribute to rhizospheric microbial activity, which plays an important role in iron acquisition (Masalha et al., 2000). Some rhizobacteria can exude a class of rhizobacteria secretion, such as antibiotics (including the antifungals), phosphate solubilization, hydrocyanic acid, indoleacetic acid (IAA), siderophores, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase which increase bioavailability and facilitate root absorption of heavy metals, such as Fe (Crowley et al., 1991) and Mn (Barber and Lee, 1974), as well as nonessential metals, such as Cd (Salt et al., 1995), enhance tolerance of host plants by improving the P absorption (Davies et al., 2001; Liu et al., 2000) and promote plant growth (Budzikiewicz, 1997; Duffy and Défago, 1999; Burd et al., 2000; Ellis et al., 2000; Meyer, 2000). Abou-Shanab et al., (2005) investigated the correlation between metal resistance and metal mobilization abilities of rhizobacteria under heavy metals stress. The highest incidence of the biochemical activity of isolates and metal resistance was recorded for: phosphate solubilizers with Cr, Zn and Pb (92.5%, 82.2% and 68.2%), respectively; then for siderophore producers with Cr, Zn and Pb (78.5%, 71.02% and 61.6%), respectively, and finally for acid producers with Cr, Zn and Pb (63.5%, 53.3% and 42.9%), respectively. This implies that phosphate solubilization is not only the mechanism adopted by bacteria towards metals in soil; but that siderophores and acid production are involved in mobilizing metals.

It should be mentioned that IAA production by rhizobacteria is believed to play an important role in plant-bacterial interactions (Lambrecht et al., 2000). Therefore, any direct influence on IAA production by bacteria may in turn affect their phytostimulating efficiency. It has been well documented that the biosynthesis of auxins with their excretion into soil makes a major contribution to the bacterial plant-growth-promoting effect (Lambrecht et al., 2000; Kamnev, 2003; Steenhoudt and Vanderleyden, 2000). It has been found that Cu2+ and Cd2+ significantly suppressed the production of IAA (auxin) by non-endophytic and facultatively endophytic strains of A. brasilense (Sp7 and Sp245, respectively), which can directly affect the plant-growth-stimulating efficiency of associative plant-bacterial symbioses in heavy-metal-polluted soils (Kamnev et al., 2005). Kamnev et al., (2005) similarly discovered that both Cu2+ and Cd2+ ions significantly decreased the level of IAA production for strain Sp7, whereas the bacterial growth rate was virtually not affected.

In addition, some plant growth-promoting bacteria i.e., free-living soil bacteria that are involved in a beneficial association with plants, contain the enzyme ACC deaminase (Glick, 1995; Glick et al., 1995; Jacobson et al., 1994), which can cleave the plant ethylene precursor ACC and lower the level of ethylene in a developing or stressed plant. Plant growth-promoting bacteria that contain ACC deaminase may act to insure that the ethylene level does not impair root growth (Glick et al., 1998), and that by facilitating the formation of longer roots, these bacteria may enhance seedling survival and plant root growth. Other properties of this bacterial strain, in addition to ACC deaminase activity, may contribute to this result: various N2-fixing and auxin-producing PGPR, siderophores, and antibiotics, all of which may stimulate plant growth in the presence of toxic metals concentrations. For example, Masalha et al., (2000) reported that plants grown under non-sterile soil systems were better in terms of iron nutrition to those grown under sterile condition. Their data emphasize the role of microbial community on the iron nutrition of plants. In fact, there is evidence that at least part of the toxic effects of some heavy metals in plants results from an induced iron deficiency, and since bacterial siderophores can provide iron to various plants (Bar-Ness et al., 1991; Reid et al., 1986; Wang et al., 1993), siderophores produced by rhizobacteria may reduce nickel toxicity by supplying the plant with iron and hence reducing the severity of nickel toxicity (Bingham et al., 1986; Bollard, 1983; Foy et al., 1978; Yang et al., 1996).

**Bioavailability of toxic heavy metals**

Soil rhizobacteria can also directly influence metal solubility by changing heavy metal speciation in the rhizosphere. Study of the roles of mycorrhiza in metal speciation in the rhizosphere and the impact on increasing host plant tolerance against excessive heavy metals in soil showed that speciations of Cu, Zn and Pb changed significantly in the rhizosphere of AM
(arbuscular mycorrhiza) infected and non-infected maize in comparison to bulk soil; The greatest change was exchangeable Cu that increased by 26 and 43% in non-infected and AM-infected rhizosphere, respectively, than in bulk soil. With the exception of organic bound Cu in AM, other speciations were stable in the rhizosphere of AM and non-AM treatments. It is understandable that Cu is more toxic to the rhizosphere than Zn or Pb. The greatest change was exchangeable Cu that increased by 26 and 43% in non-infected plants. In contrast, carbonate and Fe-Mn oxides of Zn and Pb did not exhibit significant changes. The results might indicate that mycorrhiza could protect its host plants from the phytotoxicity of excessive copper, zinc and lead by changing the speciation from bioavailable to the non-bioavailable form. The fact that copper and zinc accumulation in the roots and shoots of mycorrhiza infected plants were significantly lower than those in the non-infected plants might also suggest that mycorrhiza efficiently restricted excessive copper and zinc absorptions into the host plants (Huang et al., 2005).

Evidence has been shown that the chemical conditions of the rhizosphere differ from those of the bulk soil, as a consequence of various processes that are induced by plant roots and/or by the rhizobacteria (Hinsinger, 2001; Marschner, 1995). Plant-bacteria interactions could stimulate the production of compounds that could alter soil chemical properties in rhizosphere and enhance heavy metals accumulation in plants. For example, Delorme et al. (2001) found that soil acidification in the rhizosphere of *Thalaspi caerulescens* facilitates metal ion uptake by increasing metal ion mobility around the roots. De Souza et al. (1999b) also reported that the accumulation of Hg increases when the pH of the culture solution is lowered and hypothesized that rhizobacteria of the plants reduced the pH in the rhizosphere, thereby increasing Hg uptake into plants. A further study of the influence of hydrogen and aluminum ions on the growth of the associative nitrogen-fixing and growth-promoting bacteria *Azospirillum lipoferum* 137, *Arthrobacter myosorens* 7, *Agrobacterium radiobacter* 10, and *Flavobacterium* sp. L30 showed that the response of plants to the inoculation strongly varied from positive to negative with the soil pH (Bellmov et al., 1998). In addition, microorganisms can remove a number of metals from the environment by reducing them to a lower redox state (Lovley, 1995). Many of the microorganisms that catalyze such reactions use the metals as terminal electron acceptors in anaerobic respiration. Such microorganisms, known as dissimilatory metal-reducing bacteria, are phylogenetically (Lonergan et al., 1996) and physiologically (Lovley et al., 1997) diverse; although, most share the ability to use Fe(III) and S(II) as terminal electron acceptors (Lovley et al., 1997). The microbial reduction of Cr(VI) to Cr(III) has been one of the most widely studied forms of metal bioremediation (Lovley, 1995; Wang and Shen, 1995). A wide diversity of heterotrophic organisms is known to carry out this reaction which, depending upon the organism, can take place anaerobically or aerobically (Wang and Shen, 1995; Lovley, 1993).

Plant growth-promoting bacterial could induce resistance in plants against fungal, bacterial and viral diseases (Maurhofer et al., 1998), and insect (Zehnder et al., 1997) and nematode pests (Sikora, 1992). The induction of systemic resistance by rhizobacteria is referred to as ISR. In recent years, the use of PGPR as an inducer of systemic resistance in crop plants against different pathogens has been demonstrated under field conditions (Wei et al., 1991; 1996; Vidyasekaran and Muthamilian, 1999; Viswanathan and Samiyappan, 1999). Nie et al. (2002) reported that antibiotic-secreting plant growth-promoting bacterial strains can inhibit the proliferation and subsequent invasion of phytopathogens, hence protecting plants from further damage in the presence of arsenate. Experiments showed that seed treated with *Pseudomonas fluorescens* strain 97 protected beans against halo blight disease caused by *Pseudomonas syringae pv. phaseolicola* (Alstrom, 1991). Reports on PGPR-mediated ISR against insects are restricted to very few crops. Induction of systemic resistance by PGPR strains, viz., *Pseudomonas putida* strain 89B-27, *Serratia marcescens* strain 90-166, *Flavomonas oryzihabitans* strain INR-5 and *Bacillus pumilus* strain INR-7 have significantly reduced populations of the striped cucumber beetle, *Acalyma vittatum* and the spotted cucumber beetle, Diabrotica undecimpunctata howardi on cucumber (Zehnder et al., 1997). PGPR also induces systemic resistance against nematode pests (Oostendorp and Sikora, 1990; Sikora, 1992; Sikora and Hoffmann-Hergarten, 1992). *Pseudomonas fluorescens* has induced systemic resistance and inhibited early root penetration of *Heterodera schachtii*, the cyst nematode in sugar beet (Oostendorp and Sikora, 1989; 1990).

### Rhizobacteria secretion

As mentioned above, rhizobacteria secretion may play a major role among mechanisms of phytoremediation assisted by rhizobacteria. Indirect mechanisms include preventing phytopathogens from inhibiting plant growth and development while direct mechanisms include: nitrogen fixation; synthesis of siderophores which can solubilize and sequester iron from the soil; production of phytohormones such as auxins and cytokinins, which can enhance plant growth; and solubilization of minerals such as phosphorus (Kloeper et al., 1989; Glick, 1995; Glick et al., 1999; Patten and Glick, 1996). Rhizobacteria produce metal-chelating agents called siderophores, which have an important role in the acquisition of several heavy metals (Leong, 1986). These organic substances have the effect of scavenging Fe(III)
and significantly enhancing the bioavailability of soil bound iron (Kanazawa et al., 1994). It has also been recognized that plants grown in metal-contaminated soils are often iron deficient, the production of siderophores by plant growth-promoting bacteria may help plants obtain sufficient iron (Burd et al., 2000; Wallace et al., 1992). Microbial siderophores are used as iron chelating agents that can regulate the availability of iron in the plant rhizosphere (Bar-Ness et al., 1992; Loper and Henkels, 1999). It has been assumed that competition for iron in the rhizosphere is controlled by the affinity of the siderophore for iron and ultimately decides the rhizosphere population structure. The important factors, which participate, are concentration of various types of siderophore, kinetics of exchange, and availability of Fe-complexes to microbes as well as plants (Loper and Henkels, 1999). Interestingly, the binding affinity of phytosiderophores for iron is less than the affinity of microbial siderophores, but plants require a lower iron concentration for normal growth than do microbes (Meyer, 2000).

A number of PGPR, which stimulate root growth of different plant species including Indian mustard (Burd et al., 1998; Belimov et al., 2001), contain the enzyme ACC deaminase, which hydrolyses and decreases the amount of ACC, an ethylene precursor of the plant hormone ethylene, in plants and, as a result, to decrease ethylene biosynthesis by plants (Glick et al., 1994; 1998; Hall et al., 1996). The model which represents how a PGPR bound to either a seed or plant root lowers the ethylene concentration and thereby prevents ethylene inhibition of root elongation was previously proposed by Glick et al. (1998). In some of the plants, ACC is exuded from roots or seeds and then taken up by the bacterium and cleaved by ACC deaminase to ammonia and α-ketobutyrate (Glick et al., 1998). The bacteria utilize the ammonia evolved from ACC as a nitrogen source and thereby decrease ACC within the plant (Penrose and Glick, 2001) with the concomitant reduction of plant ethylene and promoting root elongation (Burd et al., 1998; Mayak et al., 1999; Gricichko and Glick, 2001; Belimov et al., 2002). To maintain the gradient between internal and external ACC levels, the plant must exude increasing amounts of ACC. The lowering of ACC levels within the plant results in a reduction in the amount of plant ethylene and a decreased extent of ethylene inhibition of plant seedling root elongation. This model may also be invoked to explain how plant growth-promoting bacteria lower the concentration of stress ethylene in plants. Evidence for this model includes the fact that the ability of a bacterium to promote root elongation is positively correlated with both the ACC deaminase activity of the bacterium and the ACC content (measured by high-pressure liquid chromatography) of the plant tissues.

In addition, depending on the conditions, plant root growth may also be stimulated by IAA produced by PGPR bound to the seeds or roots (Patten and Glick, 2002). As a matter of fact, low levels of IAA produced by rhizobacteria promote primary root elongation, whereas high levels of IAA stimulate lateral and adventitious root formation (Glick, 1995) but inhibit primary root growth (Xie et al., 1996). Thus, plant growth-promoting bacteria can facilitate plant growth by altering the hormonal balance within the affected plant (Glick et al., 1999). Similarly, although an ethylene pulse is important in breaking seed dormancy, too much ethylene can inhibit plant seed germination (Bewley and Black, 1985; Mayer and Poljakoff-Mayber, 1989; Small and van der Straeten, 1997). As just described above, a significant portion of the damage to plants from infection with fungal phytopathogens may occur as a direct result of the response of the plant to the increased level of stress ethylene (van Loon, 1984). In the presence of fungal pathogens, not only does exogenous ethylene increase the severity of a fungal infection but also inhibitors of ethylene synthesis can significantly decrease the severity of infection. Since the enzyme ACC deaminase, when present in plant growth-promoting bacteria, can act to modulate the level of ethylene in a plant, lower the stress placed on plants by the presence of heavy metals and therefore ameliorate some of the apparent toxicity of heavy metals to plants.

**Transform toxic heavy metals**

The efficiency of phytoremediation is also influenced by the bioavailability of metals to plants in soil. Bacteria may transform toxic heavy metals to forms that are more readily taken up into roots. For example, bacteria could enhance Se accumulation in plants by reducing selenate to organic Se, and organoselenium forms like SeMet are known to be taken up at faster rates into roots than inorganic forms (Zayed et al., 1998). Huang et al. (2005) further depicts the relative changes as the percentages of the speciation concentration difference between bulked soil and rhizosphere to the concentration of bulked soil. Results showed that the relative changes of organic bound Cu, Zn and Pb were, respectively, +5%, +23%, +3% in the infected rhizosphere, and 0.8%, −3%, and −2% in the noninfected rhizosphere. Thus, significant amounts of Cu, Zn and Pb were bounded by organic matter in the infected rhizosphere. Soil rhizobacteria can also directly influence metal bioavailability by altering their chemical properties, such as pH, organic matter content, redox state, etc. This can aid in the leaching of these contaminants from soils. The bioavailability of heavy metals in soils is a function of its solubility (Ernst, 1996) with pH and organic matter content being the main controlling factors (Gray et al., 1998). For example, a strain of *Pseudomonas maltophilia* was shown to reduce the mobile and toxic Cr⁶⁺ to nontoxic and immobile Cr⁴⁺, and also to minimize environmental mobility of other toxic ions such as Hg²⁺, Pb²⁺, and Cd²⁺ (Blake et al., 1993;...
Inhibition of plant pathogens

PGPR provides different mechanisms for suppressing plant pathogens. They include competition for nutrients and space (Elad and Baker, 1985; Elad and Chet, 1987), antibiotic production by PGPR, 1-aminocyclopropane-1-carboxylate deaminase, siderophore production, and metal solubilization. The effectiveness of such mechanisms depends on the specific pathogen and the metal content of the soil.

Stimulation of transport protein

Bacterial survival and proliferation in the environment as well as within various hosts are critically dependent on the uptake and sequestration of transition metals such as manganese, zinc, and iron. For example, cells may stringently regulate intracellular zinc levels, since high concentrations of zinc are toxic to cellular functions and have evolved several types of proteins involved in binding and transport of zinc (Claveros, 2001). Bacteria may also stimulate the sulfate transport protein, located in the root plasma membrane, which also transports selenate (Leggett and Epstein, 1956). Inorganic Hg uptake in higher plants has not been well investigated, but has been linked to the passive uptake of lipophilic chloride complexes in phytoplankton (Mason et al., 1996).

When evaluating the effect of rhizobacteria on phytoremediation in contaminated soil, regardless of the precise effects used by the bacterium to protect plants, the results from literature suggest that certain bacteria may eventually find a use in the development of phytoremediation strategies. In this regard, heavy metals may be removed from polluted soil either by increasing the metal-accumulating ability of plants or by increasing the amount of plant biomass. In heavily contaminated soil, the metal content exceeds the limit of plant tolerance, it may be possible to treat plants with plant growth-promoting rhizobacteria, increasing plant biomass and thereby stabilizing, revegetating, and remediating metal-polluted soils. However, there are many areas of poor understanding or lack of information where more research is needed. Our study demonstrated that the heavy metal-solubilizing and growth promoting bacterial strain J62 may increase the availability of the heavy metals in solution culture and the soils. Pot experiment demonstrated that the application of the bacterial strain J62 could significantly enhance maize and tomato plant biomass and heavy metal uptake. The effective heavy metal-resistant and PGPB-plant systems must be tested and established in controlled vegetation experimental designs with consideration of the specific matching of plant and microbe. This promotion of plant biomass production and heavy metal uptake by strain J62 might have potential for the phytoextraction of the metals from soils. It may therefore provide a new microbe-assisted phytoremediation of metal-polluted soils. In addition, although strain J62 significantly increased the Pb and Cd uptakes by the maize and tomato, the total Pb and Cd accumulation of the plants is low. Further understanding of the basic mechanisms of plant–microbe interactions especially hyperaccumulator-PGBP interactions is essential for Pb and Cd-contaminated soil phytoremediation.

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