Chapter 19
Role of Plant Growth Promoting Bacteria and Fungi in Heavy Metal Detoxification

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19.1 Introduction

Metals are natural parts of soils and many micronutrients including metals are required for plant growth (Sheng et al. 2008). Heavy metal ions such as Cu, Zn, Fe, Mn, Ni, and Co are essential elements, whereas Cd, Hg, Ag, and Pb are nonessential and extremely toxic elements (Williams et al. 2000; Gadd 1990). Due to industrialization and technical developments, the environment has been polluted with heavy metal by humankind (Madhaiyan et al. 2007). Unfortunately, ecosystems are still being polluted with heavy metals (Khan et al. 2000). Heavy metals are defined as elements that have an atomic numbers >20 (Jing et al. 2007) or metallic elements have specific mass higher than 5 g cm^{-3} (Gadd and Griffiths 1978). Cd, Cr, Cu, Hg, Pb, and Ni are the most common heavy metals (Jing et al. 2007).

Nowadays, metal pollution is considered as the most severe environmental problem (Jing et al. 2007; Gamalero et al. 2009; Abou-Shanab et al. 2006). Ecosystems have been contaminated with heavy metals due to different human and natural activities (Khan et al. 2000). The sources of heavy metal in the environment are: mining and smelting of metals, burning of fossil fuels, fertilizers, sewage sludge, pesticides, municipal wastes, mining, smelting, pigments, spent batteries, and vehicle exhaust (Vivas et al. 2003; Leyval et al. 1997; Denton 2007). Heavy metals are used as plant nutrients in adequate levels; however, excessive

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level of heavy metals is toxic to plants. When heavy metals are found in elevated levels in the environment, they are excessively absorbed by plant roots and transport to shoot and translocated to the shoots, where they influenced metabolism and decreased growth (Jing et al. 2007). Excessive metal concentrations in polluted soils cause a decline in soil microbial activity as well as soil fertility and yield losses (McGrath et al. 1995).

Soil-heavy metals cannot be degraded biologically but only transformed to organic complexes (Rajkumar and Freitas 2008b; Jing et al. 2007; Khan et al. 2009). Heavy metal polluted soil can be treated by chemical, physical, and biological techniques (Khan et al. 2000). In-situ techniques have some advantages such as their lower cost and reduced impact on the ecosystem compared to ex-situ techniques (Khan et al. 2000). These techniques are grouped into two different classes: ex-situ techniques (treatment on- or off-site) and in-situ techniques (treatment in-site) (Khan et al. 2000; Dary et al. 2010). Remediation methods including excavation and landfill, thermal treatment, acid leaching, and electroreclamation are not appropriate for effective remediation of heavy metals because of their high cost, low efficiency etc. (Jing et al. 2007).

Using organisms for treatment of soil pollution is termed bioremediation. An alternative method is called phytoremediation defined as "use metal accumulating plants to remove, transfer and stabilize these contaminants from soil, sediments and water" and it is a natural, clean, and economic alternative for heavy metal treatment (Khan 2005).

Metal accumulator plants are able to tolerate and concentrate high level of heavy metals. The most commonly known heavy metal accumulators are members of *Brassicaceae* family (Jing et al. 2007). *Brassica juncea* called as Indian mustard is the best-known metal-accumulator plant.

Soil can be termed as a complex interactive network (Upadhyay and Srivastava 2010; Jeffries et al. 2003) and contains a vast number of microorganisms. The rhizosphere is the site where most microorganisms thrive and is called as the largest ecosystem on earth. Bacteria, fungi, protozoa, and algae live in the rhizosphere and bacteria are the most abundant organisms among microbial communities (Ahmad et al. 2008). There is an interaction between plant and microorganisms because of rhizosphere effect. Plant root exudates many different attractive compounds into the rhizosphere for holding microbes there. Special bacteria called as plant growth promoting rhizobacteria (PGPR) that are defined as "bacteria inhabiting the rhizosphere and beneficial to plants" (Kloepper et al. 1980). PGPR is about 2–5% of rhizospheric bacteria of soil (Antoun and Prevost 2005).

Successful phytoremediation processes are required using plants, which interact with plant roots and bacteria, and studying adequate concentrations of metals in soil (Rajkumar et al. 2005). Metal-resistant microbes can help detoxification of metals in soil. Another group of microorganisms called mycorrhizae could thrive in heavy metal-contaminated soil and roots of metal accumulator plants. When mycorrhizae colonize to plant roots, it can decrease translocation of heavy metals to shoots by binding of heavy metal to the cell wall of fungal hyphae.

19.2 Heavy Metals as a Soil Pollution Agent

Heavy metals are defined as elements with metallic characteristics and an atomic number >20 (Jing et al. 2007). Heavy metals can be classified into two categories: essential (Cu, Zn, Fe, Mn, Ni, and Co) and nonessential (Cd, Hg, Ag, and Pb) elements (Williams et al. 2000; Gadd 1990). Metals are natural parts of soils (Jing et al. 2007). Heavy metal ions are main component of a variety of enzymes, transcription factors, and other proteins (Williams et al. 2000). However, excessive concentrations of both essential and nonessential heavy metals in soil can cause toxicity symptoms and inhibition of plant growth (Hall 2002). Heavy metal pollution both limits plant establishment and causes declines in numbers of soil microorganisms and their activity (Shetty et al. 1994). Also, accumulation of metals in plant organs in excessive level can limit physiological processes such as photosynthesis and synthesis of chlorophyll pigments (Wani et al. 2007b).

Influences of heavy metals on the microbial community are examined by three different ways: (1) reduction of total microbial biomass, (2) decreasing numbers of specific populations, and (3) shifting microbial community structure (Zhuang et al. 2007). Metal toxicity depends on availability, or shortly bioavailability, which refers to ability of metals from soil to living organism (Leyval et al. 1997). Factors affecting bioavailability of metals in soils are physicochemical (pH, Eh, organic matter, clay content etc.) and biological (biosorption, bioaccumulation, and solubilization) (Leyval et al. 1997). Accumulation of heavy metals by microorganisms is given in Table 19.1.

Soil-bound metals are mobilized into soil solution by three ways: (1) forming metal-chelating molecules (phytosiderophores) and releasing them into the rhizosphere to chelate and solubilize soil-bound metal, (2) decreasing soil-bound metal

Table 19.1 Accumulation of heavy metals by microorganisms (Gadd 1990)

Organism	Element	Uptake (% dry weight)
	Pb	34–40
Citrobacter sp.	Cd	13.5
Thiobacillus ferooxidans	Ag	25
Bacillus cereus	Cd	3.9–8.9
Escherichia coli	Cd	0.16–0.98
	Co	25
	Cu	34
Zoogloea sp.	Ni	13
Chlorella vulgaris	Au	10
Scenedesmus obliquus	Cd	0.3
Phoma sp.	Hg	2
	Cu	1.6
	Cd	3.0
Rhizopus arrhizus	Pb	10.4
	Cd	0.24–3.12
Saccharomyces cerevisiae	Zn	0.45

ions via plasma membrane bound metal reductases, and (3) acidifying soil environment for solubilization of metals (Raskin et al. 1994).

19.3 Phytoremediation

Heavy metal uptake by plants is a less-expensive method (Khan et al. 2000). Bioremediation is defined as "the use of organisms for the treatment of soil pollution" (Leyval et al. 2002). Phytoremediation can be defined as "the use of plants to extract, sequester and/or detoxify pollutants through physical, chemical and biological processes" (Jing et al. 2007; Glick 2003). Phytoremediation is a comparatively new approach, an environmentally friendly technique, and an emerging technology for removing pollutants from the environment (Glick 2003; Sheng et al. 2008; Garbisu and Alkorta 2001). The plants used in phytoremediation are able to tolerate and accumulate high levels of heavy metals (Nie et al. 2002). Main principles of phytoremediation are (1) extraction of pollutants from soil and translocation to shoots, (2) sequestering of pollutant via root system to prevent spreading and leaching into soil or groundwater, and (3) transformation into less toxic chemicals (Kuiper et al. 2004).

Basic phytoremediation processes are: phytoextraction, phytodegradation, rhizofiltration, phytostabilization, and phytovolatilization. Phytoremediation subgroups are defined as follows(Khan 2005): (1) phytoextraction refers to removal and concentration of metals into roots and shoot of plants, (2) phytodegradation covers degradation of contaminants by plants and their relative microbes, (3) rhizofiltration is use of plant roots for absorption of metals, (4) phytostabilization is use of plants for immobilization and reduction in mobility and bioavailability of pollutants, (5) phytovolatilization is use of plants for volatilization of pollutants from the soil into the atmosphere.

Soil contamination sources are agricultural chemicals, sewage sludge application, waste disposal, waste incineration, vehicle exhausts, anthropogenic sources etc. (Khan 2005). Using sewage sludge for agricultural purpose has been a common practice in waste disposal (Flieβbach et al. 1994). Using metals and chemicals in process industries has increasingly resulted in generation of huge amounts of effluent that contain high concentration of toxic heavy metals (Ahluwalia and Goyal 2007).

Plants that can tolerate and accumulate excessive levels of metals are defined as hyperaccumulators (Zhuang et al. 2007; Nie et al. 2002). It has been identified that approximately 400 terrestrial species are hyperaccumulators (Zaidi et al. 2006; McGrath et al. 2001). The Brassiceceae family is the most common known heavy metal accumulator (Belimov et al. 2005). Indian mustard (*Brassica juncea*) has an ability to grow in heavily contaminated soil and accumulate metals in its aboveground parts (Rajkumar et al. 2006) Hyperaccumulators are given in Table 19.2.

Plants have two main strategies for growing on metalliferous soil: (i) prevent metal from aerial part but contain high amount of metals in their root, or (ii)

Table 19.2 Some metal hyperaccumulator and their metal accumulation capacities (Lasat 2002)

Plant species	Metal	Leaf content (mg kg^{-1})
Thlaspi caerulescens	Zn, Cd	39,600–1,800
Ipomea alpine	Cu	12,300
Haumaniastrum robertii	Co	10,200
Astragalus racemosus	Se	14,900

accumulate metals in their aboveground parts (Raskin et al. 1994; Kuffner et al. 2008). Effective phytoaccumulation is dependent on two essential factors (1) having the capability of taking up and accumulating high levels of metal and (2) having the ability of producing as much biomass as possible (Burd et al. 2000; Li et al. 2007).

For adequate phytoextraction, the essential need is a phytoaccumulator that is fast growing and produces a large amount of biomass (Kamnev and Lelie 2000; Zhuang et al. 2007). Inoculation of plants with microorganisms may decrease the toxicity of heavy metals to plants in polluted soils (Madhaiyan et al. 2007). Wu et al. (2006) indicated that inoculation with PGPR may simulate plant growth and thus raise phytoremediation efficiency. Inoculation with rhizobacteria did not vastly affect the metal concentrations in plant tissues; however, the bacteria provided a much larger aboveground biomass harvest resulting in a much higher metal removal. Kumar et al. (2008) suggested that *Brassica juncea* with *Enterobacter* sp. *NBRIK28* and *Enterobacter* sp. *NBRIK28 SD1* could be used for effective phytoextraction of heavy metals from fly ash polluted sites. Nie et al. (2002) investigated the ability to proliferate in the presence of arsenate by transgenic canola with *Enterobacter cloacae UW4* ACC deaminase.

19.4 Heavy Metal Detoxification and Tolerance in Higher Plants

Potential cellular mechanisms for metal detoxification and tolerance in higher plants are summarized as (1) "restriction of metal movement to roots by mycorrhizas, (2) binding to cell wall and root exudates, (3) reduced influx across plasma membrane, (4) active efflux into apoplast, (5) chelation in cytosol by various ligands, (6) repair and protection of plasma membrane under stress conditions, and (7) transport of and accumulation of metals in vacuole" (Hall 2002).

Hyperaccumulator or accumulator plants and their related PGPR are main topic in metal detoxification. PGPR and arbuscular mycorrhizal fungi (AMF) develop plant growth and development in heavy metal polluted soil via helping root growth and branching. PGPR and AMF are able to lessen the toxicity of heavy metals either by declining the bioavailability of toxic heavy metals or raising bioavailability of nontoxic heavy metals. PGPR and AMF can alter chemical properties in the rhizosphere and stimulate metal accumulation (Denton 2007).

Menngoni et al. (2001) isolated nickel-resistant bacteria from *Alyssum bertolonii* (*Pseudomonas* and *Streptomyces*). Pseudomonas strains were located in the rhizosphere, whereas streptomyces strains were mainly found in the soil. Bacteria isolated from Alyssum murale (Ni accumulator plant) rhizosphere were *Sphingomonas macrogoltabidus*, *Microbacterium liquefaciens,* and *Microbacterium arabinogalactanolyticum* (Abou-Shanab et al. 2003). *Microbacterium oxydans AY509223* significantly increased nickel uptake by *Alyssum murale* from low, moderate, and high Ni soils (Abou-Shanab et al. 2006). Li et al. (2007) found that inoculation of *Sedum alfredii* with metal-tolerant bacterium, *Burkholderia cepacia* significantly stimulated plant growth, metal uptake in shoot, and provided better translocation of metals from root to shoot. Abou-Shanab et al. (2006) demonstrated that *Microbacterium oxydans AY509223* significantly enhanced Ni uptake of *Alyssum murale* by 36.1%, 39.3 %, and 27.7 % in low, medium, and high levels of Ni, respectively. Wu et al. (2006) investigated effects of inoculation of PGPR on metal uptake by *Brassica juncea*. They concluded that inoculation with PGPR might stimulate plant growth and thus raise phytoremediation efficiency. Wu et al. (2006) showed that presence of PGPR was very effective in protecting plants from metal inhibitory effects.

Application of Cd-solubilizing PGPR could enhance available Cd in rhizosphere soil and stimulate plant growth and Cd uptake (Sheng and Xia 2006). Rajkumar and Freitas (2008a) found that inoculation of metal-resistant bacteria (*Pseudomonas* sp. and *Pseudomonas jessenii*) to plants caused stimulation of metal accumulation in plant tissue and promoted shoot and root biomass. *Bradyrhizobium* sp.(vigna) RM8 isolated from greengram nodules reduced the uptake of Ni and Zn by plant organs (Wani et al. 2007b).

19.4.1 Fungi

In fact, it has been shown 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. Many studies about mycorrhizas have been reported in plants growing on heavy metal polluted sites. Different authors have reported most of the plants growing in heavy metal polluted sites associated with *Glomus* and *Gigaspora mycorrhizal fungal texa* (Khan et al. 2000). Weissenhorn et al. (1995) isolated *Glomus mosseae* from heavy metal polluted sites. Diaz et al. (1996) isolated *Glomus macrocarpum* from uncontaminated site, whereas *Glomus mosseae* was isolated from contaminated site.

Mycorrhizal plant metal uptake depends on several factors such as soil physicochemical properties (fertility level, pH), host plants, fungi involved, and the concentration of metal in soil (Diaz et al. 1996). Heggo et al. (1990) found that the influence of VAM fungi on heavy metal uptake is dependent upon initial soil metal concentration. Weissenhorn et al. (1994) found that heavy metals completely eliminate Arbuscular mycorrhizal (AM) colonization of plant roots in pot experiment. Gildon and Tinker (1983) found that the degree of infection with

Glomus mosseae strongly declined by adding zinc, copper, nickel, and cadmium in soil.

Fungus has an important role in detoxification that constitutes a biological barrier against transfer to the shoot. AM arbuscular mycorrhizas provide plants with essential nutrients from the soil via uptake by extraradical hyphae (Joner and Leyval 2001). Heavy metals can be transported by hyphae (Joner and Leyval 2001).

AM fungi improve plant tolerance originated heavy metal pollution (Tonin et al. 2001). Mycorrhizal fungi from uncontaminated soil provided greater biomass compared to mycorrhizal fungi from contaminated soil. Shetty et al. (1994) found that *Andropogon gerardii* only grew with the interaction of mycorrhiza in polluted soil. Mycorrhizal species such as *Suillus bovines* and *Thelephora terrestris* confined *Pinus sylvestris* against Cu toxicity (Hall 2002). Weissenhorn et al. (1995) found that the indigenous AM fungi population survived under high metal concentration in polluted soil.

Vivas et al. (2005) investigated interactive effect of *Brevibacillus brevis* and *Glomus mosseae* on plant growth. The stimulated plant Cd tolerance after coinoculation with *Brevibacillus brevis* and *Glomus mosseae* resulted in increased P and K and decreased Cd, Cr, Mn, Cu, Mo, Fe, and Ni in plant tissue.

AM fungi produce insoluble glycoprotein called glomalin that binds toxic elements such as heavy metals (Hildebrandt et al. 2007; Gonzalez-Chavez et al. 2004; Khan 2006). Glomalin can be extracted with heavy metals from soil (Gohre and Paszkowski 2006). Glomalin has a special role for filtering heavy metals at soil–hyphae interface (Khan 2006). Glomalin reduces heavy metal bioavailability via sorption and sequestration (Gonzalez-Chavez et al. 2004).

Weissenhorn et al. (1993) reported that AM infection may decline metal accumulation in plants growing in contaminated soils and thus protect the host plant against metal toxicity. Vivas et al. (2003) showed that indigenous bacteria (*Brevibacillus* sp.) coinoculated with AM fungi caused enhancing plant development under heavy metal contamination. Vivas et al. (2006) showed a protective effect of the interaction AM fungi + bacteria (*Glomus mosseae* + *Brevibacillus*) against uptake of potentially toxic Zn by Trifolium plants in moderately polluted soil. Some selected studies about mycorrhizas are given in Table 19.3.

19.4.2 Plant Growth Promoting Rhizobacteria

The rhizosphere, which is a layer of soil affected by roots and has a greater amount of microorganisms compared to surrounding bulk soil (Lugtenberg and Kamilova 2009). PGPR is defined as bacteria living the rhizosphere and beneficial to plants (Kloepper et al. 1980). PGPR improve plant health and stimulate plant growth and reduce diseases (Solano et al. 2008; Yang et al. 2009).

Inoculation of plants with microorganisms may diminish toxicity of heavy metals to plants in polluted soil (Madhaiyan et al. 2007). Bacteria are the most abundant among the other soil microbial communities (Barriuso et al. 2008) and

Table 19.3 Selected studies about mycorrhizas

Heavy metal	Mycorrhiza	Plant	Study conditions	References
Zn, Pb, Cd	*VAM*	*Andropogon gerardii*	Mine spoil	Shetty et al. (1994)
		Festuca arundinacea		
Pb	*VAM + Brevibacillus*	*Trofolium prqatense*	Pb-spiked soil	Vivas et al. (2003)
Zn, Cd, Cu, Mn, Fe	*VAM*	*Glycine max*	Zn-smelter	Heggo et al. (1990)
Zn and Pb	*Glomus mosseae*	*Lygeum sportum*	Polluted soil	Diaz et al. (1996)
	Glomus macrocorpum	*Anthyllis cytisoides*		
Zn	*AMF + Brevibacillus*	*Trifolium repens*	Zn-polluted soil	Vivas et al. (2006)
Zn, Cd	*Glomus mosseae*	*Clover*	Cd and Zn polluted soil	Tonin et al. (2001)
Cd, Ni, Cr	*Glomus mosseae*	*Cannabis sativa*	Pot experiment	Citterio et al. (2005)
Zn, Cd, Cu, Pb	*Glomus mosseae*	*Clover, maize*	Pot experiment	Joner and Leyval (2001)
Cd, Cu, Zn, Pb	*Glomus mosseae*	*Zea mays*	Pot experiment	Weissenhorn et al. (1995)

may uptake and accumulate a vast amount of metal ions (Ahluwalia and Goyal 2007). PGPR is grouped into four distinct groups which are diazototrophic PGPR, *Bacillus*, *Pseudomonas,* and *Rhizobia*. Nitrogen-fixing bacteria like *Azospirillum* sp., *Azoarcus* sp., *Burkholderia* sp., *Gluconacetobacter diazotorophic*, *Herbaspirillum* sp., *Azotobacter* sp., and *Paenibacillus polymyxa* are capable of promoting plant growth (Barriuso et al. 2008). Bacillus bacteria as defined PGPR are predominantly gram positive, whereas Pseudomonas bacteria are gram negative. Well-known PGPR are *Agrobacterium genomovars*, *Azospirillum lipoferum*, *Alcaligenes*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Methylobacterium fujisawaense*, *Pseudomonas*, *Ralstonia solanacearum*, *Rhizobium*, *Rhodococcus*, *Sinorhizobium meliloti,* and *Variovorax paradoxus* (Saleem et al. 2007).

Rhizobia show high resistance to heavy metals in symbiotic plants. *Rhizobium leguminosarum bv. Trifolii* showed up to twofold times greater resistance to Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} compared to nonmucoid colonies (Purchase et al. 1997). Wani et al. (2008) found that inoculation of *Rhizobium species RP5* affected pea plants in two different ways: (1) protecting pea plants against toxic effects of Ni and Zn and (2) decreasing the uptake of Ni and Zn by plant organs.

Khan et al. (2009) reported that different symbiotic nitrogen fixers such as *Bradyrhizobium* sp.*RM8*, *Rhizobium* sp. *RP5*, *Rhizobium* sp. *RL9,* and *Mesorhizobium* sp.*RC3* were tolerant to Ni and Zn, Ni and Zn, Zn, and Cr (VI), respectively. Nitrogen-fixing bacteria *Burkholderia* sp.*J62* was found to be heavy metal resistant (Jiang et al. 2008). There is a separation for metal resistance among

PGPR. Kuffner et al. (2008) found that *Streptomyces AR16* showed extremely high Zn resistance, whereas *Pseudomonas PR04* demonstrated low tolerance to Zn.

PGPR can be classified into two main groups based on their relationship with the plants: symbiotic bacteria and free-living bacteria (Zhuang et al. 2007; Khan et al. 2009). Free-living soil bacteria are PGPR that include a number of different bacteria such as *Azotobacter*, *Azospirillum*, *Pseudomonads*, *Acetobacter*, *Burkholderia,* and *Bacilli* (Glick et al. 1998). *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorrhizobium* are examples of symbiotic bacteria (Vessey 2003). During rhizoremediation, the plant releases root exudates that stimulate the survival and action of bacteria and finally result in more pollutant degradation (Kuiper et al. 2004). Plants eliminate and finally select bacteria beneficial and useful for their growth and health from rhizosphere (Barriuso et al. 2008). For successful plant growth-promoting inoculation bacteria must be able to rapidly settle root system during the growing season (Abou-Shanab et al. 2006). Some select studies about PGPR are given in Table 19.4.

PGPR may induce plant growth and development both directly and indirectly (Saleem et al. 2007). Indirect mechanisms happen outside the plant, whereas direct mechanisms occur inside the plant (Solano et al. 2008). PGPR can act in three different ways: (1) synthesizing special compounds for the plants, (2) facilitating uptake of some nutrients from soil, and (3) lessening or preventing the plants from pathogens (Zhuang et al. 2007; Glick et al. 1998; Khan et al. 2009; Safranova et al. 2006; Kloepper et al. 1980; Wei et al. 1996; Rapuach and Kloepper 1998; Sinha and Mukherjee 2008; Carillo-Gastaneda et al. 2002; Sharma et al. 2003). Indirect mechanisms include free nitrogen fixation, production of siderophores, phosphate solubilization, hydrolysis of molecules released by pathogens, synthesis of enzymes able to hydrolyze fungal cell walls, synthesis of cyanhydric acid, and improvement of symbiotic relationship with rhizobia and mycorrhizae (Solano et al. 2008). Direct mechanisms are given in Table 19.5.

Direct plant growth promotions are biofertilization (Vessey 2003), stimulation of root growth, rhizoremediation, and plant stress control (Lugtenberg and Kamilova 2009). PGPR can fix atmospheric nitrogen for providing plants, they can synthesize siderophores for solubilization and sequester iron from the soil and provide the plants, they can synthesize phytohormones like indolacetic acid (IAA). They may facilitate solubilization of minerals and may synthesize some enzymes (Glick et al. 1998; Rajkumar et al. 2005). PGPR can be used against plant pathogens such as *Bacillus pimulus*, *Bacillus subtilis,* and *Curtobacterium flaccumfaciens* are used as biological control against multiple cucumber pathogens (Rapuach and Kloepper 1998).

Rapuach and Kloepper (1998) investigated the effect of *PGPR stains INR7* (*Bacillus pumilus*), *GBO3* (*Bacillus subtilis*), and *ME1* (*Curtobacterium flaccumfaciens*) on multiple cucumber pathogens. The mixture of PGPR strains could stimulate disease protection and develop stability of biological control. So-Yeon and Cho (2009) found that a PGPR, *Serratia* sp. *SY5*, which could be applied as a promising microbial inoculant for the direct stimulation of plant biomass production and to indirectly enhance heavy-metal uptake by plants in the phytoremediation of heavy metal contaminated soils.

Table 19.4 Some selected studies about PGPR

PGPR	Plant	Heavy metal	References
Pseudomonas sp.		Cr^{6+}	Rajkumar et al. (2005)
Bacillus subtilis	*Brassica juncea*	Ni	Zaidi et al. (2006)
Pseudomonas sp. *M6*			Rajkumar and Freitas (2008b)
Pseudomonas jessenii M15	*Ricinus communis*	Ni, Cu, Zn	
Kluyvera ascorbata SUD165	*Brassica juncea*	Ni, Pb, Zn	Burd et al. (2000)
Enterobacter NBRI K28			
Enterobacter NBRI K28 SD1	*Brassica juncea*	Ni, Zn, Cr	Kumar et al. (2008)
Azotobacter chrooccum	*Triticum aestivum*	Cd, Cu, Ni, Zn, Pb, Cr	Athar and Ahmad (2002)
Pseudomonas sp. *NBRI4014*	*Glycine max*	Cd, Ni, Cr	Gupta et al. (2002)
Azospirillum brasilense Sp245			
Azospirillum brasilense Sp7		Co, Cu, Zn	Kamnev et al. (2005)
Pseudomonas sp. *29 C*			Rajkumar and Freitas (2008a)
Bacillus megaterium 4 C	*Brassica juncea*	Ni	
Bradyrhizobium sp *(vigna) RM8*	*Greengram*	Ni, Zn	Wani et al. (2007a)
Pseudomonas sp. *GRP3*	*Vigna radiata*	Fe	Sharma et al. (2003)
Pseudomonas asplenii	*Phragomites australis*	Cu	Reed et al. (2005)
Bacillus PSB1, PSB7, PSB10		Zn, Cr, Pb	Wani et al. (2007b)
Pseudomonas brassicacerum Am3			
Pseudomonas marginalis Dp1			Safranova et al. (2006)
Rhodococcus sp. *Fp2*	*Pisum sativum*	Cd	
Burkholderia sp. *J62*		Pb, Cd	Jiang et al. (2008)
Pseudomonas putida			
Pseudomonas sp.			
Alcaligenes xylosoxidans	*Brassica juncea*		
Alcaligenes sp.	*Brassica napus*		
Variovorax paradoxus			
Bacillus pimulus			
Rhodococcus sp.		Cd	Belimov et al. (2001)
Bradyrhizobium sp. *750*	*Lupinus luteus*	Cu, Cd, Pb	Dary et al. (2010)
Burkholderia cepacia	*Sedum alfredii*	Cd, Zn	Li et al. (2007)
Kluyvera ascorbata SUD165	*Tomato, canola, Indian mustard*	Ni, Pb, Zn	Burd et al. (2000)
Microbacterium oxydans AY509223			
Rhizobium galegae AY509213			
Microbacterium oxydans AY509219			
Clavibacter xyli AY509236			
Acidovorax avenae AY512827			
Microbacterium arabinogalactanolyticum AY5099225			
Microbacterium oxydans AY509222			
Microbacterium arabinogalactanolyticum AY509226	*Alyssum murale*	Ni	Abou-Shanab et al. (2006)
Achromobacter xylosoxidans Ax10	*Brassica juncea*	Cu	Ma et al. (2009b)
Bacillus sp. *I119*	*Rape, maize, sudangrass, tomato*	Cd	Sheng et al. (2008)
Pseudomonas tolaasii ACC23			
Pseudomonas fluorescens ACC9			
Alcaligenes sp. *ZN4*			Dell'Amico et al. (2008)
Mycobacterium sp. *ACC14*	*Brassica napus*	Cd	
Rhizobium sp. *RP5*	*Pisum sativum*	Ni, Zn	Wani et al. (2008)

Table 19.5 Direct PGPR mechanisms (Solano et al. 2008)

Mechanism	Effect
Plant growth regulator production	Biomass (aerial part and root), flowering
Ethylene synthesis inhibition	Root length
Induction of systemic resistance	Health
Root permeability raise	Biomass and nutrient absorption
Organic matter mineralization	Biomass and nutrient content
Mycorrhizal fungus association	Biomass and phosphorus content
Insect pest control	Health

The potential of bacterial inoculation is partly dependent on the original content of the heavy metals in soil. Wu et al. (2006) indicated that bacteria could be inhibited by the presence of extremely high metal concentrations, leading to a smaller population size and a decline in the activities of soil enzymes, e.g., N_2-fixing capacity and acid phosphatase activity. This may indicate that the potential of bacterial inoculation is greatest only in slightly or moderately metal-polluted sites.

19.4.2.1 Isolated Microorganisms

Many PGPR have been isolated from plants. *Bradyrhizobium* sp. *(vigna) RM8*, Ni, and Zn-tolerant PGPR were isolated from nodules of greengram (Wani et al. 2007b). Endophytic bacteria (*Staphylococcus*, *Microbacterium*, *Pseudomonas*, *Curtobacterium*, *Bacillus,* and *Arthrobacter*) were isolated and characterized from nickel hyperaccumulator plant *Alyssum bertolonii* (Barzanti et al. 2007). Endophytic bacteria (*Elsholtzia splendens* and *Commelina communis*) were isolated from tolerant plants (Sun et al. 2010). *Bacillus* sp*., strain EB1* was isolated from sites that were polluted with heavy metal in the southeast region of Turkey (Yilmaz 2003). Metal-tolerant PGPR, *NBRI K28 Enterobacter* sp., was isolated from fly ash contaminated soils (Kumar et al. 2008).

Characterization of bacterial communities from heavy metal contaminated soils has been widely studied. These kinds of studies showed that excessive levels of heavy metal could influence both the qualitative and quantitative structure of microbial communities with declining metabolic activity and biomass as well as suppressed diversity (Menngoni et al. 2001). Ganesan (2008) selected *Pseudomonas aeruginosa strain MKRh3* for rhizoremediation of cadmium soil. Similarly, Tripathi et al. (2005) isolated and characterized siderophore-producing lead and cadmium-resistant *Pseudomonas putida KNP9*. Trivedi et al. (2007) demonstrated chromate-reducing bacteria isolated from Himalayan Region named *Rhodococcus erythropolis MtCC 7905*.

19.4.2.2 ACC Deaminase Activity (ACC:1-Aminocyclopropane-1-Carboxylic Acid)

Dell'Amico et al. (2008) demonstrated that PGPR having ACC deaminase activity was critical for the growth of *Brassica napus*. Saleem et al. (2007)

reported that inoculation with PGPR and having ACC deaminase activity could be useful in maintaining plant growth and development under stressed conditions by decreasing stress-induced ethylene production. Inoculation with PGPR having ACC deaminase and final physiological changes in plants is given in Table 19.6.

Belimov et al. (2001) isolated 15 bacterial strains containing ACC deaminase from the rhizosphere of pea (*Pisum sativum*) and Indian mustard (*Brassica juncea*).These strains are *Pseudomonas brassicacearum*, *Pseudomonas marginalis*, *Pseudomonas oryzihabitans*, *Pseudomonas putida*, *Pseudomonas* sp, *Alcaligenes xylosoxidans*, *Alcaligenes* sp., *Variovorax paradoxus*, *Bacillus pumilus*, and *Rhodococcus* sp. They concluded that the beneficial effect of this kind of PGPR on plant growth significantly varied depending on features of the bacterial strain, plant genotype, and on the growth conditions, which may influence the ethylene status of the plant. The results suggest that PGPR containing ACC deaminase offer promise as bacterial

Table 19.6 Inoculation with PGPR having ACC deaminase and final physiological changes in plants (Saleem et al. 2007)

Plant species	PGPR	Comments	References
Brassica compestris	*Methylobacterium fujisawaense* *Bacillus ciculans DUC1* *Bacillus firmus DUC2* *Bacillus globisporus DUC3*	Bacterium induced root elongation Bacterial inoculation stimulated root and shoot elongation	Madhaiyan et al. (2007) Ghosh et al. (2003)
Brassica napus	*Alcaligenes* sp. *Bacillus pumilus* *Pseudomonas* sp. *Variovorax paradoxus*	Promoted growth compared uninoculated treatment	Belimov et al. (2001)
Diathus caryophyllus L.	*Azospirillum brasilense*	Longest roots	Li et al. (2005)
Pisum sativum L.	*Rhizobium leguminosarum bv.vicae 128C53K*	Stimulated nodulation	Ma et al. (2003)
Vigna radiate L.	*Pseudomonas* sp. *Bradyrhizobium* sp.	Enhanced nodulation	Shaharoona et al. (2006)
Vigna radiate L.	*Pseudomonas putida*	Ethylene production was inhibited in inoculated cuttings	Mayak et al. (1999)
Zea mays L.	*Enterobacter sakazokii 8MR5* *Pseudomonas* sp. *4MKS8* *Klebsiella oxytoca 10MKR7*	Inoculation elevated agronomic properties of maize	Bababia et al. (2003)
Zea mays L.	*Pseudomonas* sp.	Bacterium provided root elongation	Shaharoona et al. (2006)

inocula for the improvement of plant growth, particularly under unfavorable environmental conditions.

ACC is the immediate precursor of ethylene in plants and ACC deaminase metabolizes ACC to α-ketobutyrate and ammonia (Grichko et al. 2000). *Agrobacterium rhizogenes* containing ACC deaminase was helpful to plants for increasing tolerance to nickel via inhibiting ethylene production (Stearns et al. 2005). *Phychrobacter* sp. *SRA1*, *SRA2*, *Bacillus cereus SRA10*, *Bacillus* sp. *SRP4,* and *Bacillus wehenstephanensis SRP12* containing ACC deaminase, nickel-resistant bacteria, were isolated from *Alyssum serpyllifolium* and *Phleum phleoides* (Ma et al. 2009a). Madhaiyan et al. (2007) found that the bacterial strains *CBMB20* and *CBMB40* tolerate the presence of high concentrations of $NiCl_2$/$CdCl_2$ in the growth media and bind considerable amounts of Ni and Cd, in part due to the PGPR containing the enzyme ACC deaminase, which can act to alter the level of ethylene in plants.

The ability of *V. paradoxus* to use ACC as both N and C sources gives these bacteria an additional competitive advantage in rhizosphere colonization. Belimov et al. (2005) suggested that taking into account that Cd induces plant stress ethylene biosynthesis and probably contributes to accumulation of ACC in roots, it is not surprising that a number of *V. paradoxus* strains were isolated among bacterial communities dominating the root zone of *B. juncea* grown in contaminated soils in their study.

19.4.2.3 Ethylene

Ethylene is a plant hormone and has vast importance in plant growth and development. Production of ethylene significantly increases under stress conditions (Belimov et al. 2001). Increased ethylene levels in plants shows exposure to various types of stress such as chilling, heat, wounding, drought, flooding, pathogen infection, and nutritional stress (Stearns et al. 2005).

Actually, ethylene is required by many plants for seed germination. However, excessive ethylene causes suppression of root elongation. PGPR with ACC deaminase promotes root elongation in a variety of plants and using of ACC deaminase that contains PGPR decreases the level of stress ethylene (Glick 2003). Heavy metals can stimulate ethylene production by plants and an excess of ethylene can inhibit plant development (Burd et al. 1998).

In the presence of excessive metals, most plants either synthesize stress ethylene or become severely depleted in iron (Glick 2003). Madhaiyan et al. (2007) showed that *Methylobacterium oryzae strain CBMB20* and *Burkholderia* sp. *Strain CBMB40* from rice decreased Ni and Cd toxicity and promoted plant growth by reducing stress ethylene. Some PGPR produce ACC deaminase that inhibits synthesis of ethylene (So-Yeon et al. 2010). Rodecap and Tigey (1981), and Fuhrer (1982) indicated that among heavy metals, cadmium (Cd) is the strongest inductor of ethylene biosynthesis in plants.

19.5 Protecting Metal Inhibitory Effect

Burd et al. (2000) mentioned that *Kluyvera ascorbata SUD 165/26* protected plants against the inhibitory effects of high levels of nickel, lead, and zinc. Sinha and Mukherjee (2008) indicated that using Cd-resistant bacterial strain provided reduction of Cd uptake in plants. Ma et al. (2009a) noted that inoculation of metal-resistant serpentine strains (*Phychrobacter* sp. *SRA1*, *SRA2*; *Bacillus cereus SRA10*; *Bacillus* sp. *SRP4*; *Bacillus weihenstephanensis SPR12*) showed to be very effective in protecting plants from Ni toxicity. Another study were studied by the same researchers investigated plant growth promoting bacterium *Achromobacter xylosoxidans strain Ax10* for improvement of copper phytoextraction by *Brassica juncea*. *A. xylosoxidans Ax10* protected plant from Cu toxicity and stimulated Cu accumulation in plant tissue (Ma et al. 2009b). *Kluyvera ascorbata SUD165/26* decreased heavy metal toxicity (Ni, Pb, and Zn) in plants (Burd et al. 2000). Ma et al. (2009b) reported that inoculation of metal-resistant serpentine strains (*Psychrobacter* sp. *SRA2*, *SRA1*, *Bacillus cereus SRA10*) effectively protected plants (*Brassica juncea* and *Brassica oxyrrhina*) from growth inhibition due to Ni. Rajkumar et al. (2006) found *Pseudomonas* sp. *PsA4* and *Bacillus* sp. *Ba32* protect plants (*Brassica juncea*) against negative effect of chromium.

Zaidi et al. (2006) indicated that inoculation of *Bacillus subtilis strain SJ-101* not only confined plant from Ni toxicity but also stimulated Ni accumulation in plant tissue with enhancement of plant growth. Burd et al. (2000) found *Kluyvera ascorbata SUD165* protected plants (tomato, canola, and Indian mustard) against negative effects of high concentrations of Ni, Pb, and Zn. Reed et al. (2005) found that inoculation of *Phragmites australis* seeds with *Pseudomonas asplenii AC* and *P. asplenii AC-1* develop seed germination and plant growth and partially defend plants from inhibition by copper. Dell'Amico et al. (2008) indicated that cadmium-resistant rhizobacteria (*Pseudomonas tolaasii ACC23*, *Pseudomonas fluorescens ACC9*, *Alcaligenes* sp. *ZN4,* and *Mycobacterium* sp. *ACC14*) protected plants (*Brassica napus*) against the inhibitory effects of cadmium possibly due to production of IAA, siderophores, and ACC deaminase activity. Wani et al. (2008) found similar results that inoculation *Rhizobium species RP5* resulted in protection to the pea plants against toxic effects of Ni and Zn. Also they noted the reduced uptake of nickel and zinc by plant organs.

19.6 Conclusions

Nature has the ability to refresh itself by various techniques. Environmental pollution, especially heavy metal pollution, can be dealt with by the activities of microorganisms through heavy metal detoxification. The most common detoxification microorganisms are bacteria PGPR, and fungi AMF. Results from the literature suggest that heavy metals may be removed from contaminated soils using PGPR

and AMF by assisting root growth and branching. Interactions with detoxification microorganisms and hyperaccumulator plants may be the future solution for detoxification of metals in soils.

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References

Abou-Shanab RAI, Angle JS, Delorme TA, Chaney RL, Berkum P, Moavad H, Ghanem K, Ghozlan HA (2003) Rhizobacterial effects on nickel extraction from soil and uptake by *Alyssum murale*. New Phytol 158:219–224

Abou-Shanab RAI, Angle JS, Chaney RL (2006) Bacterial inoculants affecting nickel uptake by *Alyssum murale* from low, moderate and high Ni soils. Soil Biol Biochem 38:2882–2889

Ahluwalia SS, Goyal D (2007) Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour Technol 98:2243–2257

Ahmad I, Pinchtel J, Hayat S (2008) Plant–bacteria interactions. Weinheim, Wiley-VCH

Antoun H, Prevost D (2005) Ecology of plant growth promoting rhizobacteria in PGPR. In: Siddiqui ZA (ed) Biocontrol and biofertilization. Springer, The Netherlands, pp 1–39

Athar R, Ahmad M (2002) Heavy metal toxicity effect on plant growth and metal uptake by wheat, and on free living Azotobacter. Water Air Soil Pollut 138:165–180

Bababia OO, Osir EO, Sanni AI, Odhaimbo GD, Bulimo WD (2003) Amplification of 1-aminocyclopropane-1-carboxylic (ACC) deaminase from plant growth promoting rhizobacteria in striga-infested soils. Afr J Biotechnol 2:157–160

Barriuso J, Solano BR, Lucas JA, Lobo AP, Garcia-Villanaco A, Monero FJG (2008) Ecology, genetic diversity and screening strategies of plant growth promoting rhizobacteria. In: Ahmad I, Pichtel J, Hayat S (eds) Plant–bacteria interactions. Weinheim, Wiley-VCH, pp 1–13

Barzanti R, Ozino F, Bazzicalupo M, Gabbrielli R, Galerdi F, Gonelli C, Mengoni A (2007) Isolation and characterization of endophytic bacteria from the nickel hyperaccumulator plant *Alyssum bertolonii*. Microb Ecol 53:306–316

Belimov AA, Safranova VI, Sergeyeva TA, Egorova TN, Matueyeva VA, Tsyganov VE, Borisov AY, Tikhanovich IA, Kluge C, Preisfeld A, Dietz K, Stepanok VV (2001) Characterization of plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-caboxylate deaminase. Can J Microbiol 47:642–652

Belimov AA, Hontzeas N, Safranova VI, Demchinskoya SV, Piluzza G, Bullitta S, Glick BR (2005) Cadmium tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (Brassica juncea L.Czern). Soil Biol Biochem 37:241–250

Burd GI, Dixon DG, Glick BR (1998) A plant growth-promoting bacterium that decreases nickel toxicity in seedlings. Appl Environ Microbiol 64(10):3663–3668

Burd GI, Dixon DG, Glick BR (2000) Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46:237–245

Carillo-Gastaneda G, Munos JJ, Peralta-Videa JR, Gomez E, Tiemann KJ, Duarte-Gardea M, Gardea-Torresdey JL (2002) Alfalfa growth promotion by bacteria grown under iron limiting conditions. Adv Environ Res 6:391–399

Citterio S, Prato N, Fumagalli P, Aina R, Massa N, Santagostino A, Sgorbati S, Berto G (2005) The arbuscular mycorrhizal fungus *Glomus mosseae* induces growth and metal accumulation changes in *Cannabis sativa* L. Chemosphere 59:21–29

Dary M, Chamber-Perez MA, Palomeres AJ, Pajuela E (2010) "In situ" phytostabilisation of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. J Hazard Mater 177:323–330

Dell'Amico E, Cavalca L, Andreoni V (2008) Improvement of *Brassica napus* growth under cadmium stress by cadmium-resistant rhizobacteria. Soil Biol Biochem 40:74–84

Denton B (2007) Advances in phytoremediation of heavy metals using plant growth promoting bacteria and fungi. Basic Biotechnol J 3:1–5

Diaz G, Azcon-Aguilar C, Honrubia M (1996) Influence of arbucular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of *Lygeum spartum* and *Anthyllis cytisoides*. Plant Soil 180:241–249

Flieβbach A, Martens R, Reber HH (1994) Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biol Biochem 26(9):1201–1205

Fuhrer J (1982) Ethylene biosynthesis and cadmium toxicity in leaf tissue of beans (*Phaseolus vulgaris* L.). Plant Physiol 70:162–167

Gadd GM (1990) Heavy metal accumulation by bacteria and other microorganisms. Experientia 46:834–840

Gadd GM, Griffiths AJ (1978) Microorganisms and heavy metal toxicity. Microb Ecol 4:303–317

Gamalero E, Lingua G, Berta G, Glick BR (2009) Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Can J Microbiol 55:501–514

Ganesan V (2008) Rhizoremediation of cadmium soil using a cadmium-resistant plant growth-promoting Rhiopseudomonad. Curr Microbiol 56:403–407

Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Bioresour Technol 77(3):229–236

Ghosh S, Penterman JN, Little RD, Chavez R, Glick BR (2003) Three newly isolated plant growth-promoting bacilli facilitate the seedling growth of canola, *Brassica compestris*. Plant Physiol Biochem 41:277–281

Gildon A, Tinker PB (1983) Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants: I. The effects of heavy metals on the development of vesicular-arbusculat mycorrhizas. New Phytol 95:247–261

Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393

Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28:367–374

Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J Theor Biol 190:63–68

Gohre V, Paszkowski U (2006) Contribution of arbuscular symbiosis to heavy metal phytoremediation. Planta 223:1115–112

Gonzalez-Chavez MC, Carillo-Gonzalez R, Wright SF, Nichols KA (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollut 130:317–323

Grichko UP, Filby B, Glick BR (2000) Increased ability of transgenic plants expressing the bacterial enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, Pb and Zn. J Biotechnol 81:45–53

Gupta A, Meyer JM, Goel R (2002) Development of heavy metal-resistant mutants of phosphate solubilizing *Pseudomonas* sp. NBRI4014 and their characterization. Curr Microbiol 45:323–327

Hall JL (2002) Celular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53(366):1–11

Heggo A, Angle JS, Chaney RL (1990) Effects of vesicular-arbuscular mycorrhizal fungi on heavy metal uptake by soybeans. Soil Biol Biochem 22(6):865–869

Hildebrandt U, Regvar M, Bothe H (2007) Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68:139–146

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea J (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 37:1–16

Jiang C, Sheng X, Qian M, Wang Q (2008) Isolation and characterization of a heavy metal-resistant *Burkholderia* sp. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil. Chemosphere 72:157–164

Jing Y, He Z, Yang X (2007) Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. J Zhejiang Univ Sci B 8(3):192–207

Joner EJ, Leyval C (2001) Time-course of heavy metal uptake in maize and clover as affected by root density and different mycorrhizal inoculation regimes. Biol Fertil Soils 33:351–357

Kamnev AA, Lelie D (2000) Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. Biosci Rep 20(4):239–258

Kamnev AA, Tugarova AV, Antonyuk LP, Tarantilis PA, Polissiou MG, Gardiner PHE (2005) Effects of heavy metals on plant-associated rhizobacteria: comparison of endophytic and non-endophytic strains *Azospirillum brasilense*. J Trace Elem Med Biol 19:91–95

Khan AG (2005) Role of soil microbes in the rhizospheres of plant growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364

Khan AG (2006) Review: mycorrhizoremediation-an enhanced form of phytoremediation. J Zhejiang Univ Sci B 7(7):503–514

Khan AG, Kuek C, Chaundhry TM, Khoo CS, Hayes WJ (2000) Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197–207

Khan MS, Zaidi A, Wani PA, Oves M (2009) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem Lett 7:1–19

Kloepper JW, Schroth MN, Miller TD (1980) Effect of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70(11):1078–1082

Kuffner M, Puschenreiter M, Wieshammer G, Gorfer M, Sessitsch A (2008) Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating willows. Plant Soil 304:35–44

Kuiper I, Lagendijk EL, Bloomberg GV, Lugtenberg BJJ (2004) Rhizoremediation: a beneficial plant–microbe interaction. Mol Plant Microbe Interact 17(1):6–15

Kumar KV, Singh N, Behl HM, Srivastava S (2008) Influence of plant growth promoting bacteria and its mutant on heavy metal toxicity in *Brassica juncea* grown in fly ash amended soil. Chemosphere 72:678–683

Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31:109–120

Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7:139–153

Leyval C, Joner EJ, del Val C, Haselwandter K (2002) Potential of arbuscular mycorrhizal fungi for bioremediation. In: Gianinazzi S, Schhuepp H, Barea JM, Haselwanter K (eds) Mycorrhiza technology in agriculture. Birkhauser Verlag Basel, Switzerland, pp 175–186

Li Q, Saleh-Lakka S, Glick BR (2005) The effect of native and ACC deaminase-containing *Azospirillum brasilense* Cd843 on the rooting of cornation cuttings. Can J Microbiol 51:511–514

Li WC, Ye ZH, Wong MH (2007) Effects of bacteria on enhanced metal uptake of the Cd/Zn-hyperaccumulating plant, *Sedum alfredii*. J Exp Bot 58(15/16):4173–4182

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting bacteria. Annu Rev Microbiol 63:541–556

Ma W, Guinel FC, Glick BR (2003) *Rhizobium leguminosarum* biovar viciae 1-amino-cyclopropane-1-carboxylate deaminase promotes nodulation of pea plants. Appl Environ Microbiol 69:4396–4402

Ma Y, Rajkumar M, Freitas H (2009a) Improvement of plant growth and nickel uptake by nickel resistant-plant-growth promoting bacteria. J Hazard Mater 166:1154–1161

Ma Y, Rajkumar M, Freitas H (2009b) Inoculation of plant growth promoting bacterium *Achromobacter xylosoxidans* strain Ax10 for the improvement of copper phytoextraction by *Brassica juncea*. J Environ Manage 90:831–837

Madhaiyan M, Poonguzhali S, Sa T (2007) Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (*Lycopersicon esculentum* L.). Chemosphere 69:220–228

Mayak S, Tivosh T, Glick BR (1999) Effect of wild type and mutant plant growth-promoting rhizobacteria on the rooting of mungbean cuttings. J Plant Growth Regul 18:49–53

McGrath SP, Chaundri AM, Giller KE (1995) Long-term effects of metals in sewage sludge on soils, microorganisms and plants. J Ind Microbiol 14(2):94–104

McGrath S, Zhao FJ, Lombi E (2001) Plant and rhizosphere processes involved in phytoremediation of metal-contaminated soils. Plant Soil 232:207–214

Menngoni A, Barzanti R, Gonnelli C, Gabrielli R, Bazzicalupo M (2001) Characterization of nickel-resistant bacteria isolated from serpentine soil. Environ Microbiol 3(11):691–698

Nie L, Shah S, Rashid A, Burd GI, Dixon DG, Glick BR (2002) Phytoremediation of arsenate contaminated soil by transgenic canola and the plant growth-promoting bacterium *Enterobacter cloacae* CAL2. Plant Physiol Biochem 40:355–361

Purchase D, Miles RJ, Young TWK (1997) Cadmium uptake and nitrogen fixing ability in heavy-metal-resistant laboratory and field strains *of Rhizobium leguminosarum* biovar trifolii. FEMS Microbiol Ecol 22:85–93

Rajkumar M, Freitas H (2008a) Effects of inoculation of plant-growth promoting bacteria on Ni uptake by Indian mustard. Bioresour Technol 99:3491–3498

Rajkumar M, Freitas H (2008b) Influence of metal resistant-plant growth-promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals. Chemosphere 71:834–842

Rajkumar M, Nagendran R, Lee KJ, Lee WH (2005) Characterization of a novel Cr^{6+} reducing *Pseudomonas* sp. with plant growth-promoting potential. Curr Microbiol 50:266–271

Rajkumar M, Nagendran R, Lee KJ, Lee WH, Kim SZ (2006) Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard. Chemosphere 62:741–748

Rapuach GS, Kloepper JW (1998) Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88(11):1158–1164

Raskin I, Nanda Kumar PBA, Dushenkov S, Salt DE (1994) Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5:285–290

Reed MLE, Warner BG, Glick BR (2005) Plant growth-promoting bacteria facilitate the growth of the common reed *Phragmites australis* in the presence of copper or polycyclic aromatic hydrocarbons. Curr Microbiol 51:425–429

Rodecap KD, Tigey DT (1981) Stress ethylene: a bioassay for rhizosphere-applied phytotoxicants. Environ Monit Assess 1:119–127

Safranova VI, Stepanok VV, Engqvist GL, Alekseyev YV, Belimov AA (2006) Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. Biol Fertil Soils 42:267–272

Saleem M, Arshad M, Hussain S, Bhatti AS (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Ind Microbiol Biotechnol 34:685–648

Shaharoona B, Arshad M, Zahir ZA (2006) Performance of *Pseudomonas* spp. containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. Soil Biol Biochem 38:2971–2975

Sharma A, Johri BN, Sharma AK, Glick BR (2003) Plant growth-promoting bacterium *Pseudomonas* sp. strain GRP3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). Soil Biol Biochem 35:887–894

Sheng X, Xia J (2006) Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium resistant bacteria. Chemosphere 64:1036–1042

Sheng X, He L, Wang Q, Ye H, Jiang C (2008) Effects of inoculation of biosurfactant-producing *Bacillus* sp. J119 on plant growth and cadmium uptake in a cadmium-amended soil. J Hazard Mater 155:17–22

Shetty KG, Hetrick BAD, Figge DAH, Schwab AP (1994) Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. Environ Pollut 86:181–188

Sinha S, Mukherjee SK (2008) Cadmium-induced siderophore production by a high Cd-resistant bacterial strain relevied Cd toxicity in plants through root colonization. Curr Microbiol 56:55–60

Solano BR, Maicas JB, Monero FJG (2008) Physiological and molecular mechanisms of plant growth promoting rhizobacteria (PGPR). In: Ahmad I, Pichtel J, Hayat S (eds) Plant–bacteria interactions. Weinheim, Wiley-VCH, pp 41–52

So-Yeon K, Cho K (2009) Isolation and characterization of a plant growth-promoting rhizobacterium, *Serratia* sp. SY5. J Microbiol Biotechnol 19(11):1431–1438

So-Yeon K, Hong SH, Ryu HW, Cho K (2010) Plant growth-promoting trait of rhizobacteria isolated from soil contaminated with petroleum and heavy metals. J Microbiol Biotechnol 20(3):587–593

Stearns JC, Shah S, Greenberg BM, Dixon DG, Glick BR (2005) Tolerance of transgenic canola expressing 1-aminocyclopropane-1-carboxylic acid deaminase to growth inhibition by nickel. Plant Physiol Biochem 43:701–708

Sun L, Zhang Y, He L, Chen Z, Wang Q, Qian M, Sheng X (2010) Genetic diversity and characterization of heavy metal-resistant-endophytic bacteria from two copper-tolerant plant species on copper mine wasteland. Bioresour Technol 101:501–509

Tonin C, Vandenkoornhuyse P, Joner EI, Straczek J, Leyval C (2001) Assesment of arbucular mycorrhizal fungi diversity in the rhizosphere of viola calaminaria and effect of these fungi on heavy metal uptake by clover. Mycorrhiza 10:161–168

Tripathi M, Munot HP, Shouche Y, Meyer JM, Goel R (2005) Isolation and functional characterization of siderophore-producing lead and cadmium resistant *Pseudomonas putida* KNP9. Curr Microbiol 50:233–237

Trivedi P, Pandey A, Sa T (2007) Chromate reducing and plant growth promoting activities of psychrotrophic *Rhodococcus erythropolis* MtCC 7905. J Basic Microbiol 47:513–517

Upadhyay A, Srivastava S (2010) Evaluation of multiple plant growth promoting traits of an isolate of *Pseudomonas fluorescens* strain Psd. Indian J Exp Biol 48:601–609

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Vivas A, Azcon R, Biro B, Barea JM, Ruiz-Lozano JM (2003) Influence of bacterial strains isolated from lead-polluted soil and their interactions with arbuscular mycorrhizae on the growth of *Trifolium pratense* L. under lead toxicity. Can J Microbiol 49:577–588

Vivas A, Barea JM, Azcon R (2005) Interactive effect of *Brevibacillus brevis* and *Glomus mosseae* both isolated from Cd contaminated soil on plant growth, physiological mycorrhizal fungal characteristics and soil enzyme activities in Cd polluted soil. Environ Pollut 134:257–166

Vivas A, Biro B, Ruiz-Lozano JM, Barea IM, Azcon R (2006) Two bacterial strains isolated from a Zn-polluted soil enhance plant growth an mycorrhizal efficiency under Zn-toxicity. Chemosphere 62:1523–1533

Wani PA, Khan MS, Zaidi A (2007a) Chromium reduction, plant growth-promoting potential, and metal solubilization by *Bacillus* sp. isolated from alluvial soil. Curr Microbiol 54:237–243

Wani PA, Khan MS, Zaidi A (2007b) Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp. (vigna) on growth, symbiosis, seed yield and metal uptake by greengram plants. Chemosphere 70:36–45

Wani PA, Khan MS, Zaidi A (2008) Effect of metal-tolerant plant growth-promoting rhizobium on the performance of pea grown in metal-amended soil. Arch Environ Contam Toxicol 55:33–42

Wei G, Kloepper JW, Tuzun S (1996) Induced systemic resistance to cucumber diseases and increased plant growth. Biol Control 86(2):221–224

Weissenhorn I, Leyval C, Berthelin J (1993) Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy metal polluted soils. Plant Soil 157:247–256

Weissenhorn I, Glashoff A, Leyval C, Berthelin J (1994) Differential tolerance to Cd and Zn of arbuscular mycorrhizal (AM) fungal spores isolated from heavy metal-polluted and unpolluted soils. Plant Soil 167:189–196

Weissenhorn I, Leyval C, Belgy G, Berthelin J (1995) Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. Mycorrhiza 5:245–251

Williams LE, Pittman JK, Hall J (2000) Emerging mechanisms for heavy metal transport in plants. Biochim Biophys Acta 1465:104–126

Wu CH, Wood TK, Mulchandani A, Chen W (2006) Engineering plant–microbe symbiosis for rhizoremediation of heavy metals. Appl Environ Microbiol 72(2):1129–1134

Yang J, Kloepper JW, Ryu C (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14(1):1–4

Yilmaz EI (2003) Metal tolerance and biosorption capacity of *Bacillus circulans* strain EB1. Res Microbiol 154:409–415

Zaidi S, Usmani S, Singh BR, Musarrat J (2006) Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. Chemosphere 64:991–997

Zhuang X, Chen J, Shim H, Bai Z (2007) New advances in plant growth-promoting rhizobacteria for bioremediation. Environ Int 33:406–413