Chapter 14
Potential for the Use of Rhizobacteria in the Sustainable Management of Contaminated Soils

Vincenza Andreoni and Patrizia Zaccheo

Abstract The removal of contaminants from the environments has become a crucial problem that requires a variety of approaches to reach suitable solutions. This review will focus on the use of rhizobacteria for restoration of sites co-contaminated with organic pollutants and heavy metals. While the first contaminants can be biodegraded to innocuous end products, metals are not biodegradable and must either be removed or stabilized within the site. Plant growth promoting rhizobacteria (PGPRs) represent a wide variety of soil bacteria which, when grown in association with a host plant result in stimulation of growth of their host also in a stressed environment. Plants, especially dicotyledons that are treated with ACC deaminase-containing PGPRs are more resistant to the deleterious effects of ethylene synthesized as a consequence of stressful conditions. In this review the use of PGPRs to assist plants in remediation processes is examined by discussing recent advances in bioaugmentation efforts. The effectiveness of the external manipulation of rhizosoil to overcome physical and chemical constraints to root establishment and to enhance pollutant removal is also examined. Finally, it is provided a summary of the recent advances in the potential for the use of transgenic plants and/or microorganisms to remediate environmental contaminants. The complexity and diversity of plant/soil/microorganism systems require an integrated approach involving basic and applied researches in order to establish phytoremediation as a viable and attractive technology for efficient restoration of co-contaminated soils

Keywords Rhizoremediation · Plant tolerance · Plant growth promoting rhizobacteria · Detoxification genes · ACC deaminase activity

V. Andreoni (✉)
Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università Degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy
e-mail: vincenza.andreoni@unimi.it

P. Zaccheo (✉)
Dipartimento di Produzione Vegetale, Università Degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy
e-mail: patrizia.zaccheo@unimi.it

M. Ashraf et al. (eds.), *Plant Adaptation and Phytoremediation*,
DOI 10.1007/978-90-481-9370-7_14,

Contents

1 Introduction

Rapid industrialization coupled with increased urbanization and changing agricultural practices have resulted in the non-judicious production and use of chemical compounds. Consequently, the environment has become heavily contaminated with pollutants that are toxic to both the environment and human health. Many sites are currently co-contaminated with organic pollutants and heavy metals. Therefore, the removal of contaminants has become a crucial problem that requires a variety of approaches to reach suitable solutions.

Phytoremediation, which is the use of plants to remove pollutants or to render them harmless through physical, chemical and biological processes (Cunningham and Ow 1996; Pilon-Smits 2005), is a low-cost and ecologically accepted technology for *in situ* decontamination of soil and water. During phytoremediation the soil biological properties and physical structure are maintained and soil fertility and biodiversity can be improved. Moreover, well-planted phytoremediation site prevent landscape destruction while garnering strong public support due to the aestetic appearance of the plants. As shown in Fig. 14.1, phytoremediation includes different processes, among which rhizoremediation and phytoextraction represent more challenging techniques for remediating soil that has been contaminated with organic and inorganic pollutants. Additionally, microbe-assisted phytoremediation has recently been employed by exploiting the symbiotic plant-microbe relationship in a rhizosphere (Chaudhry et al. 2005; Gerhardt et al. 2006). Plant roots provide a large surface area for a large population of bacteria and transport the colonizing bacteria to a depth of 10–15 m in the soil. During rhizoremediation, the root system distributes microorganisms through the soil and penetrates otherwise-impermeable soil layers while drawing soluble forms of the pollutants in the soil water phase towards the plant and the microorganisms. Moreover, the plant roots help increase the availability of the pollutant by breaking apart and aerating soil particles as well as by pumping water to the root-colonizing bacteria which helps improve their survival.

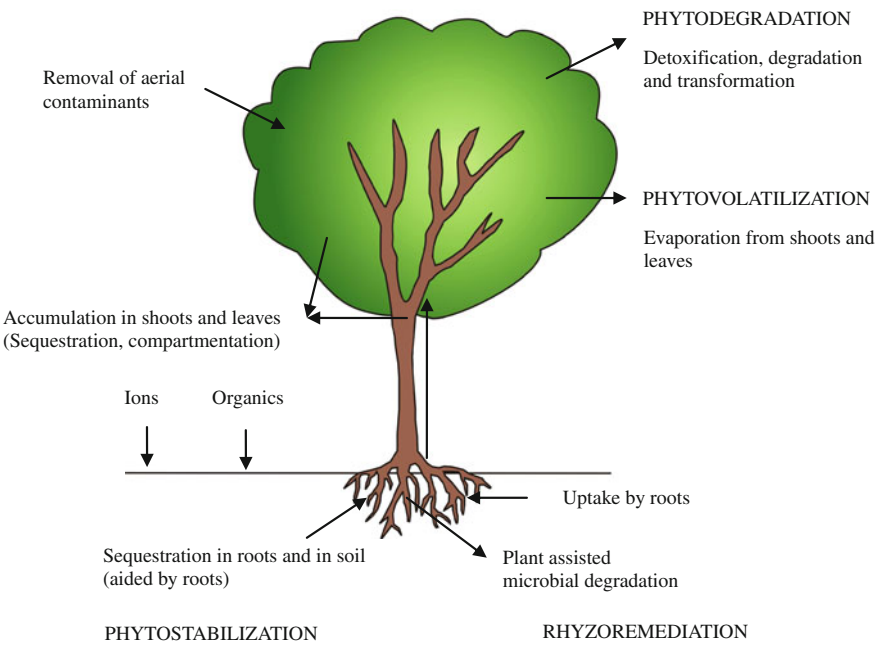

Fig. 14.1 Mechanisms involved in inorganic and organic pollutant decontamination / degradation in phytoremediation processes

Xenobiotic pollutants that can be remediated/metabolised include thrichloroethylene (TCE), polychlorinated biphenyls (PCBs), pesticides, explosives, trinitrotoluene (TNT), petroleum hydrocarbons (PHC), polycyclic aromatic hydrocarbons (PAHs) and detergents (Macek et al. 2000; Newman and Reynolds 2004). Soils that have been contaminated by weathered hydrocarbons and heavy metals (Palmroth et al. 2006) have been effectively treated with rhizoremediation. Processes involved in the phytoremediation of xenobiotic pollutants are microbial transformation and/or mineralization and plant uptake, translocation, transformation and compartmentalization of the contaminants. Rhizosphere factors play an important role in phytoremediation efficiency during successful rhizoremediation projects. Indeed, N- and P-fertilizers, root exudation and chelating agents can enhance plant uptake and accumulation of contaminants by improving the availability of the pollutants to the plants.

Some naturally occurring plants, known as hyperaccumulators have the potential to bioconcentrate metals to 10–500 times higher than non-accumulator species do. Despite this capacity, most hyperaccumulator plants are not suitable for field phytoremediation due to their small biomass production (Shen and Liu 1998).

2 Fate of Contaminants in the Rhizosphere

Once introduced into soil, organic and inorganic contaminants interact with the soil solid phase through many chemical, physical and biological processes (sorption/desorption, precipitation/dissolution, microbial immobilization/ mineralization). As shown in Table 14.1, pH, redox and dissolved organic matter play a fundamental role in controlling the fate and bioavailability of inorganic pollutants (Kabata-Pendias 2004).

PAHs tend to be strongly adsorbed to soil colloids, particularly organic matter, and the hydrophobicity of PAHs result in their having a high persistence in soil. Additionally, xenobiotics can undergo to an ageing process or be sequestered with time in microsites, which result in their becoming more tightly sorbed and less bioavailable (Ruggiero et al. 2002). In the rhizosphere, PAHs are strongly adsorbed to the roots, and this effect is more pronounced with increasing plant age (Schwab et al. 1998).

Table 14.1 Bioavailability of inorganic pollutants under different soil conditions

Condition		**redox**		**pH**	
		low reducing	*high* oxidizing	*low* acid	*medium-high* neutral-alkaline
Increasing metal mobility ↑	*high*	As	Zn	Zn, Cu, Co, Ni, Hg	
	medium		Cu, Co, Cd, Ni	Cd	Cd
	low		Pb	Pb	Pb
	very low	Cu,Co,Ni, Zn, Hg, Cd, Pb	Fe, Mn, Al, Sn, Cr		Cu, Co, Ni, Zn, Hg

Rhizospheric soil has chemical, physical and biological properties that are quite different from bulk soil due to the root activity and the presence of free enzymes and rhizobacteria (Hinsinger et al. 2003). In the rhizosphere, the mobility of heavy metals and redox sensitive elements such as arsenic (As), copper (Cu) and mercury (Hg) may increase greatly, leading to the contamination of crop plants. For example, a sixfold increase in bioavailable Cu in the rhizosphere of maize grown in a fungicide polluted soil was reported by Cattani et al. (2006). However, little Cu uptake by maize occurred, presumably due to the sequestration of Cu by dissolved organic carbon (DOC), which was present in the rhizosphere in levels three-fold greater than that of bulk soil. Enhancement of soluble Ni driven by the formation of Ni-organic

complexes and the dissolution of Ni-bearing minerals through ligands was observed in the rhizosphere of Ni hyperaccumulator plants (Krämer et al. 1996; Wenzel et al. 2003). The ability of *Pteris vittata* L. to hyperaccumulate arsenic is related to a fern-mediated increase in rhizosferic soil pH of 0.4 units and a DOC concentration of 33–40% (Silva-Gonzaga et al. 2006). However, *Thlaspi caerulescens* L., which is a well known Zn hyperaccumulator plant, does not mobilize Zn through soil acidification and root exudation (Luo et al. 2000; Zhao et al. 2001; Whiting et al. 2001).

Also soil microorganisms can modify chemical properties of the rhizospheric soil, thus affecting inorganic contaminant bioavailability. While bacteria may enhance the ion bioavailability by exuding a variety of organic compounds or stimulating the release of exudates by the plants (Salt et al. 1995), mycorrhizae may reduce metal phytoavailability by sequestering these compounds in the hyphae (Lasat 2002).

3 The Interactions Among Bacteria and Organic and Inorganic Pollutants

Organic-degrading microorganisms and a number of metal-resistant microorganisms that are known to detoxify metals/metalloids have been isolated from impacted soils and characterized (Daane et al. 2001; Singer et al. 2004; Cavalca et al. 2004; Dell'Amico et al. 2008). Bacteria degrade xenobiotics through a variety of enzymes including peroxidases, monooxygenases and dioxygenases, laccases, phosphatases, dehalogenases, nitrilases, and nitroreductases (Siciliano et al. 2001; Gibson and Parales 2000; Gianfreda and Rao 2004; Andreoni and Gianfreda 2009).

Although some microorganisms can completely degrade a specific xenobiotic, individual species generally do not contain entire degradation pathways. Rather, microbial consortia in the rhizosphere work synergistically to effectively degrade the pollutants (Chaudhry et al. 2005; Yateem et al. 2007). For example, the synergistic degradation of naphthalene by two *Pseudomonas fluorescens* strains in the rhizosphere of a grass was reported by Bloemberg et al. (2000). Moreover, by labelling the strains with different autofluorescent protein markers, the authors observed the frequency of the appearance and distribution of pure and mixed microcolonies along the root and found that mixed colonies only occurred in the presence of naphthalene, presumably because one strain secreted naphthalene intermediates that were used by the other strain when they were close to each other on the root.

It is also becoming clear that the horizontal transfer of genes plays a large role in the spread of functional abilities within communities and in enabling the adaptation of organisms to changing niches by allowing the acquisition of new metabolic potential for degradation of recently introduced xenobiotics (Janssen et al. 2005; Phale et al. 2007) or for detoxification of inorganic pollutants.

Genes located on chromosomes, plasmids or transposons encode specific resistance to a variety of inorganic elements. The most frequent mechanism of arsenic,

cadmium and mercury resistance is the energy dependent pumping out of these compounds, via membrane efflux pumps. Prominent examples include inducible plasmid-encoded resistance for Cd by the *cad* operon in *S. aureus* and *Bacillus sp.* or by the *czc* operon found in *Alcaligenes eutrophus* (Nies 2003), as well as resistance for Hg encoded by the *mer* operon found in Gram-negative and Gram-positive bacteria (Barkay et al. 2003) and resistance for As, and antimonite (Sb) mediated by the *ars* operon in *E. coli* (Rosen 2002) and *S. aureus* (Messens et al. 1999). Each *ars* operon has two essential components: the arsenate reductase (arsC, ACR2) and an arsenite-specific efflux pump (ArsB, ACR3) (Silver and Phung 2005). Although arsenic resistance is not directly involved in arsenate respiration and arsenite oxidation, *ars* operons have been found in arsenate-respiring bacteria (Saltikov and Newman 2003) as well as and in many arsenite-oxidizing bacteria, providing the latter the ability to both oxidize and reduce arsenic (Macur et al. 2004). The *Mer* operon generally contains a mercuric reductase (merA), but in some organisms the operon also contain an organonomercurial lyase (merB) that cleaves certain organomercuric compounds (Barkay et al. 2003). An overview of membrane associated uptake, efflux, reduction and oxidation of the cited ions is reported in Fig. 14.2.

While organic contaminants can be biodegraded to innocuous end products (CO_2, cell mass, water), metals are not biodegradable and must either be removed or stabilized within the site.

Co-contaminated soils, which are widespread throughout the world, are still considered difficult to remediate due to the mixed nature of the contaminants (Sandrin and Maier 2003). The presence of metals can impact both the physiology and ecology of organic degrading microorganisms. Metals may inhibit pollutant degradation through interaction with enzymes involved in biodegradation (e.g., pollutant-specific oxygenases) or with enzymes involved in general metabolism (Angle and Chaney 1989).

Metal toxicity is related to the concentration of bioavailable ionic species rather than the total metal concentration. Usually, inhibition of biodegradation increases progressively as the concentration of bioavailable metal in a co-contaminated environment increases. When considering the impact of metals on organic biodegradation, the effects of metals on populations other than degraders of the parent compound must be also considered. Reduced microbial activity may also originate from changes in the microbial community structure after long-term exposure to heavy metals. Doelman et al. (1994) observed that metal-contaminated soil contained more metal-resistant microorganisms, but with a restricted ability to degrade organic pollutants. The presence of multiple contaminants may present extreme challenges to the maintenance of a phylogenetically and functionally diverse microbial community. In soils contaminated with both heavy metals and hydrocarbons, only those that tolerate both contaminants may survive. Shi et al. (2002) when examined microbial community composition and activity after long-term exposure to Pb, Cr, and hydrocarbons, found that the soil microbial community was not affected by metals but predominantly by hydrocarbons.

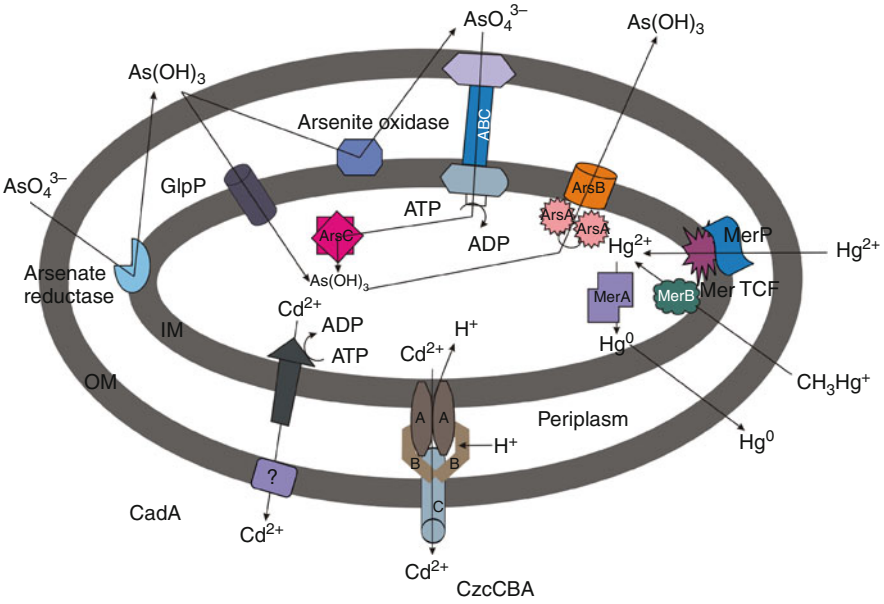

Fig. 14.2 Overview of membrane associated uptake, efflux, reduction and oxidation of arsenic, mercury and cadmium. For cadmium, a schematic presentation of the efflux systems is given due to the complexity of CadA P-type ATPases and chemiosmotic CzcCBA systems. Arsenic and mercury are given more emphasis, as peryplasmic and cytoplasmic enzymes are included as well as the class of transporters. GlpP, aquaglycerolporine; ABC, multicomponent Pst-like ATPase uptake system; ArsA/B, two component ATPase efflux pump; ArsC, small intracellular arsenate reductase; MerP, peryplasmic protein that binds Hg^{2+}; MerT/C/F, alternative membrane uptake proteins; MerB, organomercurial lyase; MerA, mercuric reductase; CzcCBA, three polypeptide chemiosmotic complex that function as an ion/proton exchanger to efflux Cd^{2+}; CadA, P-type membrane efflux ATPase for Cd^{2+} (large single polypeptide) (Adapted from Silver Phung 2005; Barkay et al. 2003; Nies 2003)

The influence of heavy metals on PAH degradation in polluted soils has recently been emphasized, and the effect of various metals on the degradation of phenanthrene has been thoroughly investigated. The degradation of phenanthrene was found to be retarded by the presence of Cu, and high levels of the metal caused incomplete mineralization and accumulation of phenanthrene metabolites (Sokhn et al. 2001). A marginal stimulation of the phenanthrene biodegradation rate in soil occurred when 140 mg kg^{-1} phenanthrene was in the presence of 40 mg kg^{-1} Zn. However, phenanthrene degradation was inhibited at Zn concentrations at or above the "action" values (i.e., the level of a contaminant at which soil quality is deemed to impair the soil functional properties) (Wong et al. 2005).

Stimulated biodegradation at low metal concentrations and inhibition at high metal concentrations has also been observed. The addition of hexavalent chromium (0.01 ppm total chromium) was found to increase the biodegradation rate of phenol by 177% and that of benzoate of 169% over controls without metals (Kuo and

Genthner 1996). Similar results were obtained by Hughes and Poole (1989). These responses suggested that the stimulatory effect could be due to metals competition for reducing equivalents or nutrients between metal-resistant degrading bacteria and non degrading bacteria that are metal-sensitive. However, Roane and Pepper (1997) found that a population of 2,4-D degrading bacteria in a Cd contaminated soil showed higher resistance at 40 mg L^{-1} than at 20 mg/L and that the higher Cd concentration inhibited less the biodegradation. This response can be explainable by microbial community dynamics wherein high metal concentrations create selective pressure for metal-resistant degraders. Specifically, a reduction in the competition of metal-sensitive non degrading microorganisms may have led to increased biodegradation at higher metal concentrations.

Dual bioaugmentation appears to be a viable approach in the remediation of co-contaminated soils. A dual bioaugmentation that employed metal-detoxifying and organic-degrading bacteria to remove 2,4-D from co-contaminated soils in the laboratory and a pilot field experiment was found to be effective (Roane et al. 2001). The success of the bioremediation strategy, which required a 48-hour time interval between inoculation with a cadmium-detoxifying population of bacteria (*Pseudomonas* spp. H1) and inoculation with a cadmium sensitive 2,4-degrader (*Ralstonia eutropha* JMP 134), was attributed to metal detoxification as the primary mode of bacterial action, which resulted in organic degradation no longer being inhibited. Indeed, some microbial mechanisms of resistance to metal, such as metal sequestration and precipitation, can reduce the toxicity toward organic degrading microorganisms.

Aerobic degradation of TCE can occur through many different oxygenases, including toluene *ortho*-monooxygenase (TOM) (Mars et al. 1996). The stable integration of the *TOM* gene of *Burkholderia cepacia* G4 into naturally occurring rhizobacteria that had colonized the roots of a poplar tree such as *Pseudomonas* Pb2-1 and *Rhizobium* strain 1032D was found to enable the establishment of a bacterium-plant-soil microcosm in which 63% of the TCE was degraded in 4 days (Shim et al. 2000). The subsequent introduction of a gene coding for the metal-binding peptide EC20 in the Pb2-1 and 10320D strains gave rise to strains with both metal accumulation (extracellularly) and TCE degradation capabilities (Lee et al. 2006). Thus, the bioaugmentation of the rhizosphere with a microorganism that is capable of both organic degradation and metal resistance may represent another means of bioremediation.

4 Rhizospheric Microbial Populations

The rhizosphere is an area encircling the plant root system that is characterized by enhanced microbial biomass productivity. Rhizobacteria obtain nutrients excreted from roots, such as organic acids, amino acids, enzymes and complex carbohydrates. The enhanced growth of microorganisms also depends on microenvironmental conditions (chemical factors, pH, O_2 content and redox potential).

In return, rhizobacteria that promote plant growth (PGPR) convert nutrients into available minerals for the plants, synthesize compounds that protect the plants against stress hormone levels and plant pathogens, and degrade and/or immobilize contaminants before they can negatively impact the plants (Hontzeas et al. 2004; Chaudhry et al. 2005; Liu et al. 2007). PGPR are fast-growing bacteria that include numerous genera such as *Bacillus*, *Pseudomonas*, *Erwinia*, *Flavobacterium, Caulobacter, Serratia, Arthrobacter, Comamonas, Alcaligenes, Agrobacterium* and free-living nitrogen fixing bacteria (Gray and Smith 2005). Among these bacteria, *Pseudomonas* spp. predominate rhizosperic soil and discontinuously colonize root surfaces, resulting in random distribution on roots. For example, *P. putida* are species that respond rapidly to the presence of root exudates in soil, converging through chemotaxis and motility mechanisms at root colonization sites, where they establish stable biofilms (Broek and Venderleyden 1995; Espinosa-Urgel et al. 2002). Numerous bacterial traits, such as production of thiamine and biotin, synthesis of the O-antigen of lipopolysaccharide and cellulose, production of amino acids and the presence of an efflux pump induced by isoflavonoids are required for effective root colonization. Flavonoids and coumarins are an important group of plant compounds that are structurally similar to many xenobitics such as PCBs, PAHs, and PHC, thereby stimulating the growth and activity of PHC, PAH and PCB degrading bacteria (Chaudhry et al. 2005; Leigh et al. 2006).

The successful application of rhizoremediation is largely dependent on the capacity of degrading bacteria or PGPR to efficiently colonize growing roots. Moreover, many PGPR play an important role in metal solubilisation, which is a prerequisite for rhizoremediation and/or phytoremediation, by producing indoleacetic acid or metal-chelating compounds such as siderophores that release metal cations from soil particles (Khan 2005) and thus favour metal uptake.

5 Methods for Assessing and Monitoring Rhizospheric Bacteria

It is essential to thoroughly understand the role that bacteria play in phytoremediation to maximize the sustained bioremediation that occurs under natural conditions and to monitor the presence, survival and activity of degrading or detoxifing micro-organisms. Until recently, studies of *in situ* bioremediation were primarily based on cultivation techniques. However, pure culture isolation, biochemical testing using methods such as BIOLOG and counting methods (plate counts or most probable number, MPN) are not well suited for the estimation of total microbial biomass or the assessment of community composition within environmental samples. Accordingly, culture-independent methods, that rely on the isolation of signature biomarkers, such as DNA, RNA and phospholipid fatty acid (PLFA) have been used to provide a quantitative measure of the rhizosphere microbial biomass, community composition, nutritional status, relative frequency of specific functional genes and, in some cases, the community metabolic activity. PLFA provides a broad-scale diversity index that can be used to evaluate the number of bacterial families

present in the samples. Additionally, combinations of BIOLOG and PLFA have been used to demonstrate differences in the microbial composition of bulk and rhizospheric soil (Soderberg et al. 2004). However, these methods are inadequate to describe the abundance and diversity of microbial communities in the environment or to relate a microbial species to the ecosystem function, but these limitations can be overcome by using a number of culture-independent approaches.

Polymerase chain reaction (PCR) to amplify selected fragments of DNA isolated from soil microorganisms or environmental DNA samples, combined with fingerprinting techniques, such as ribosomal intergenic spacer analysis (RISA), denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment analysis (T-RFLP), amplified rDNA restriction analysis (ARDRA), cloning and sequencing can provide detailed information about the species composition of communities (Spiegelman et al. 2005). As a result, detection of specific nucleic acid sequences and nucleic acid hybridization, using specific probes for a functional gene involved in a degradation pathway (i.e., *nah* and *nod* gene sequences encoding for naphthalene dioxygenase and *phen* gene sequences for phenanthrene dioxygenase) or for metal resistance genes, or gene messages, are indispensable for the identification of microorganisms in environmental samples for the evaluation of their bioremediation potential. For example, PAH-degrading bacteria have been detected and characterized in salt marsh rhizospheres using a variety of phenotypic and molecular properties (Daane et al. 2001). In a total of five different plant samples, the primary bacterial groups were Gram-negative pseudomonads, Gram-positive (predominantly nocardioform), and the Gram-positive, spore forming group, *Paenibacillus*. Furthermore, 75% of the pseudomonad isolates hybridized to the classical *nah* gene from *P. putida* NCIB9816-4, while approximately the same number hybridized to the *nag* genes cloned from *C. testosterone* GZ42, whereas the *Paenibacillus* isolates were not found to be homologous with any of the tested gene probes (Daane et al. 2001). Siciliano et al. (2001) observed that naphthalene dioxygenase (*ndoB)* catabolic genotypes were enriched in the rhizosphere of *Scirpus pungens* in response to pollution in a contaminant-dependent manner.

DGGE has been used to demonstrate that different plants supported different bacterial, archeabacterial and fungal communities (Griffiths et al. 2003; Nicol et al. 2003; Gomes 2003). Furthermore, the addition of Hg^{2+} to a silt loam was found to cause an increase in the abundance of two RISA bands that were subsequently identified as a *Clostridium*-like organism and a *Ralstonia*-like organism (Ranjard et al. 2000). However, it is important to note that these techniques result in destruction of the samples.

To study the pattern of microbial plant-root colonization, microscopy, microscopy combined with the use of marked strains, or strains equipped with reporter genes can be used. Reporter technology has been used to assess several functions in the rhizospheric soil including gene expression even at the single cell level. The increasing knowledge of the promoter and regulator genes along with the refinement of reporter gene insertion techniques will allow to use this technique for monitoring induction, expression and regulation of virtually any

gene in the rhizosphere (Jansson 2003). The *Gfp* and *lux* genes are examples of common reporter genes that encode green fluorescent protein (GFP) or bioluminescence, respectively, and can be used to tag environmental bacteria with degrading or detoxifying capabilities. The visual reports of *gfp* and *lux* can be assayed non-destructively, without supplying external cofactors or substrates to cells. For example, *Comamonas* sp. strain CNB-1 isolated from an activated sludge and capable of degrading 4-chloronitrobenzoate (4-CNB) was applied for the rhizoremediation of 4-CNB-polluted soil through association with the alfalfa plant (Liu et al. 2007). The inoculation of CNB-1 in the rhizosphere was evaluated by constructing a GFP-expressing strain CNB-1: *gfp2* and then monitoring the colonization of alfalfa roots by CNB-1: *gfp2* and the formation biofilms on the surface and within roots by confocal laser scanning microscopy. Additionally, a *Pseudomonas fluorescens* F113rifpcb bioreporter, utilizing a chlorobenzoate-responsive promoter was used to monitor the cell-activity in alfalfa rhizospheric soil contaminated by PCBs. In particular, the fluorescence-emitting cells of the modified bacterium F113rifpcb were found to be located in microcolonies, occurring all along the root (Boldt et al. 2004). Finally, Tom-Peterson et al. (2001) determined the amount of Cu bioavailable in a soil amended with complex organic material using a specific Cu reporter construct harboured by an indigenous soil bacterium, *P. fluorescens* DF57.

One drawback of techniques based on probes is that investigations are limited to the identification of known groups and may fail to capture the presence of truly novel organisms. Fluorescent *in situ* hybridization (FISH) allows the phylogenetic identification of uncultured bacteria in natural environments using fluorescent group specific phylogenetic probes targeting rRNA and fluorescence microscopy. Combining FISH with microautoradiography or with immunodetection of bromodioxyuridine allows the detection and quantification of the active population utilizing a specific substrate (Cottrell and Kirchman 2000; Pernthaler and Amann 2004).

The extraction and characterization of mRNA from soil can provide data on activity of certain genotypes in polluted soils. Naphthalene degradation for example has been monitored by quantification of mRNA transcripts of naphthalene dioxygenase gene (*nahAC)* (Sanseverino et al. 1993–1994). Microarrays are increasingly being used to analyse microbial communities (phyogenetic oligonucleotide array), to characterize microorganisms in environmental samples and to monitor gene expression under different growth conditions (functional genes and expression arrays) (Zhou 2003). For example, Mark et al. (2005) used DNA microarrays to identify unique *P. aeruginosa* genes expressed during growth in artificial medium containing sugarbeet exudates from two beet cultivars.

Stable isotope probing (SIP), which involves tracking of a stable isotope atom from a substrate into components of microbial cells, provides phylogenetic and functional information, such as lipid content and DNA and RNA sequences. Butler et al. (2003) reported the use of PLFA-SIP to reveal spatial and temporal differences in microorganisms utilizing root exudates in the rhizosphere of ryegrass. More recently, Rangel-Castro et al. (2005) applied RNA-SIP to a $^{13}CO_2$-pulsed labelled grassland microbial community to determine the effect of liming on the structure of the rhizosphere microbial community metabolizing root exudates.

6 PGPR with ACC Deaminase Activity

PGPR are free-living saprophytic bacteria that inhabit the plant rhizosphere and colonize the root system. PGPR have long been used as plant growth promoters to increase agricultural production and as biocontrol agents against plant diseases (Zehnder et al. 2001). Recently, the application of PGPR has been extended to the remediation of contaminated soils in association with plants due to their catabolic versatility, excellent root colonizing ability and the capacity to produce a wide range of enzymes and metabolites that favour the plants under varied stress conditions (Ramamoorthy et al. 2001; Mayak et al. 2004).

For many plants, a burst of ethylene is required to break seed dormancy; however, following germination, a sustained high level of ethylene would inhibit root elongation. In addition, ethylene is synthesized in plant tissues from the precursor 1-aminocyclopropane-1-carboxylic acid (ACC) during biotic and abiotic stress conditions, which can depress growth and causes senescence in crop plants (Ma et al. 2003). Many PGPR strains and some fungi possess the enzyme ACC deaminase (Shah et al. 1998; Glick et al. 2007) which can cleave the plant ethylene precursor ACC, thereby lowering the level of ethylene in a developing seedling or stressed plant. The gene encoding ACC deaminase has been found in a variety of soil bacteria (Glick 2003; Madhaiyan et al. 2007; Dell'Amico et al. 2008; Saravanakumar and Samyappan 2007) and more than one type of ACC deaminase gene may exist (Shah et al. 1998; Babalola et al. 2003; Blaha et al. 2006). Plants, especially dicotyledons that are treated with ACC deaminase-containing PGPR are dramatically more resistant to the deleterious effects of stress ethylene synthesized as a consequence of stressful conditions. The formation of longer roots through the action of ACC deaminase may facilitate the survival of plant seedlings under various stress conditions, such as flooding (Grichko and Glick 2001), phytopathogens (Wang et al. 2000), drought and high salt concentration (Mayak et al. 2004), and heavy metals (Grichko et al. 2000). For example, ACC deaminase rhizobacteria have the potential to protect canola and tomato seeds from Ni toxicity (Burd et al. 1998) and Indian mustard, rape and canola from Cd toxicity (Belimov et al. 2005; Dell'Amico et al. 2008).

Prolific root growth may also enhance the rates of rhizoremediation. For example, a multi-component phytoremediation system of soil that combined land farming, bio-augmentation with PAH-degrading bacteria and the growth of plants (*Festuca arundinacea*) with PGPR containing ACC deaminase activity under laboratory conditions led to improved effective removal of 16 persistent and soil-bound PAHs, when compared to the results of treatment with any of these methods alone (Huang et al. 2004). Phytoremediation was successful because the plant species were able to grow in the presence of high levels of contaminants and the strains of PGPR increased plant tolerance to PAHs and accelerated plant growth in heavily contaminated soils.

Liu et al. (2007) demonstrated that the inoculation of alfalfa with *Comamonas* sp. CBN-1 eliminated the phytotoxicity of 4-CNB by completely removing it from soil within 1 or 2 days. However, the presence of ACC deaminase activity in this

bacterium was not investigated. Besides the role that ACC deaminase activity plays in alleviating ethylene-mediated stresses, the addition of other traits, the ecology of the bacterium and the physiology of the plant may also have interacted with the plant system to increase resistance to stress.

To date, very little work has been conducted to evaluate the use of ACC deaminase containing bacteria in rhizoremediation of organic-contaminated soil. Wu et al. (2006) also found that the inoculation of sunflower roots with the engineered rhizobacterium, *P. putida* 06909, caused a marked decrease in Cd phytotoxicity and a 40% increase in Cd accumulation in the roots. However, they did not investigate the ACC deaminase activity of the bacterium. A comparison of the efficiency of transgenic bacteria that carry ACC deaminase and control bacteria at promoting seed germination and root elongation in soils contaminated by copper and PAHs revealed that both native and transformed *Pseudomonas asplenii* AC equally promoted seed germination and root elongation under stress conditions (Reed and Glick 2005). Moreover, the efficiency of transgenic inoculated strains was found to be affected by soil pH, temperature, moisture content and competition with native microflora and microfauna.

Additionally, according to Burd et al. (1998) and Belimov et al. (2005), PGPRs containing ACC deaminase have great potential for use in the development of bacterial inocula for improvement of plant growth under unfavourable environmental conditions, particularly for hyperaccumulator plants. Furthermore, the plant growth promotion observed in response to inoculation with ACC deaminase-containing bacteria has been found to stimulate the development of transgenic plants that express ACC deaminase genes, thus protecting them from some of the deleterious effects of metals.

7 Plant Tolerance to Toxic Compounds and Transgenic Plants with Detoxification Genes

The potential for the use of transgenic plants and/ or microorganisms to remediate environmental contaminants has been extensively explored in the laboratory. Strategies feasible for the transformation and engineering of microorganisms or plants include the introduction of genes encoding functions to enhance resistance to contaminants or to environmental stressors, to overexpress enzymes involved in degradation pathways, to release specific exudates that can act as inducers for microbial degradation, and to increase the plant capacity for the uptake, transport and sequestration of contaminants. For example, for metal phytoremediation purposes transgenic plants may be manufactured to synthesize a product that alters metal tolerance or uptake that decreases ethylene synthesis to reduce the deleterious plant response to metal stress.

Transgenic tomato plants and transgenic canola plants expressing bacterial ACC deaminase were found to grow in soil in the presence of cadmium, copper, cobalt, nickel, lead or zinc, to accumulate high amounts of metals and to proliferate in the

presence of high levels of arsenate (Grichko et al. 2000; Nie et al. 2002). In the presence of arsenate, transgenic canola plants grew to a significantly greater extent than non-transformed canola plants, regardless of whether plant growth-promoting bacteria were present. Additionally, plants accumulated similar amounts of arsenate whether or not they were treated with *E. cloacae* CAL2. Moreover, transgenic canola shoots contained less arsenate than non-transformed canola shoots, suggesting that a limited translocation of arsenate from roots to shoots occurred, which may have lowered arsenate toxicity, even if the reason for this decreased translocation in transgenic plants was unknown. When biomass was considered in calculating the arsenate accumulation, transgenic canola plants accumulated approximately four times as much arsenate as non-transformed canola. The higher rate of germination of transgenic canola also contributed to the total amount of arsenate accumulation. The use of transgenic canola in conjunction with plant growth-promoting bacteria made phytoremediation much more efficient (Nie et al. 2002). Similar results were reported by Stearns et al. (2005) and Farwell et al. (2006) in the phytoremediation of nickel contaminated soils.

Meagher and Heaton (2005) evaluated the capability of *Arabidopsis* transgenic plants expressing bacterial metal resistance genes (*merA, merB and arsC*) to take up and transform levels of mercury and arsenic several times higher than the lethal level for most plant species. *Mer* plants, which are modified plants expressing the bacterial *merB* gene encoding an organomercury lyase, were found to grow on

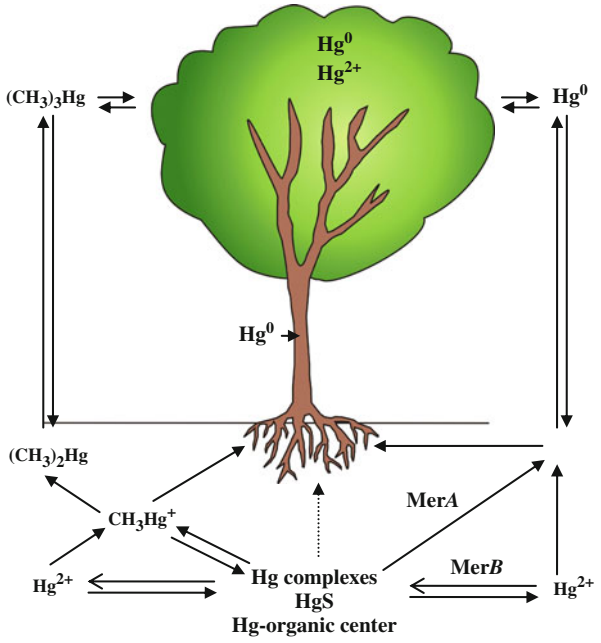

Fig. 14.3 Role of microorganisms and plants in the biogeochemical cycle of Hg

0.1–1 μM of methylmercury or phenylmercuryacetate in agar medium, which are levels high enough to kill native plants. Plants expressing the *merA* gene which encodes mercuric reductase, detoxify Hg^{2+} by reducing it to Hg^0, allowing the plants to grow in soil containing concentrations of Hg^{2+} of 100 ppm or higher (Rugh et al. 1996). Finally, combining the transgenic expression of *merA* and *merB*, enabled plants to detoxify organic mercury more efficiently and to be resistant to 2–10 μM of phenylmercuryacetate (Bizily et al. 1999; Bizily et al. 2003).

In theory, plants engineered with both genes should extract organomercurials from soils and transpire Hg^0 into the atmosphere using the same mechanisms as bacteria (Fig. 14.3). However, from a regulatory perspective, the release of Hg into the atmosphere is not acceptable, therefore, the use of plants genetically transformed with *merA* and *merB* gene is not permitted.

8 Strategies for Enhancing Phytoremediation

Studies of *in situ* application of rhizoremediation have provided contradictory results because several biotic and abiotic factors may severely limit the establishment of vegetation, microbial growth and contaminant mobility. The ability of plants to enhance rhizospheric activity and to extract contaminants from the soils can be drastically reduced by contaminant phytotoxicity or by unsuitable soil physical and chemical properties such as acidity, compaction, and anoxic conditions. The consequent reductions in root development represent severe constraints in phytoremediation because contaminants are often heterogeneously located in soil and a limited root system cannot gain access to niches with a high degree of pollution. These constraints can be partially overcome by selecting tolerant plants, and/or by applying agronomic techniques to amend soil properties and modify contaminant bioavailability. For example, nitrogen and phosphorus fertilization increased the rhizobacteria-assisted phytoextraction of As (Jankong et al. 2007), as well as pyrene rhizodegradation (Thompson et al. 2008). Additionally, soil amendments with humified organic matter enhanced the biodegradation of PCBs (Smith et al. 2007) and of aged hydrocarbons and heavy metals in co-contaminated soils (Palmroth et al. 2006)

There is evidence that the external manipulation of bulk and/or rhizospheric soil pH can improve the phytoremediation of metal polluted soils in cases of low metal concentration in soil solution due to strong binding to the solid phases. Conversely, it is still a matter of debate if plants can transfer high amounts of metals from soil into the shoots by adopting rhizosphere strategies such as acidification and exudation. A decrease in bulk soil pH can be achieved through application of mineral acids, organic acids and acid-producing fertilizers (Cui et al. 2004; Kayser et al. 2001). Acidification of the rhizosphere may be obtained by modulating the nitrogen nutrition, and supplying $N\text{-}NH_4$ to plants has been found to induce rhizosphere acidification, thereby enhancing Cd and Zn uptake by tobacco and

sunflowers (Loosemore et al. 2004; Zaccheo et al. 2006). Conversely, a N-NO_3 supply promoted growth and phytoextraction of Cd and Zn by *Thlaspi caerulescens* (Xie et al. 2009).

There is evidence that organic acids released from the roots of some plants can provide the impetus for movement of PAHs from bulk soil to the rhizosphere and accelerate PAH mobilization (Liste and Alexander 2000). Root exudation of chelators may be mimicked by the addition of natural and synthetic compounds (i.e., citric acid, NTA) to enhance heavy metal solubility and phytoextraction efficiency of several plant species like willow, Indian mustard, corn and sunflower (Schmidt 2003). It is, however, important to minimize the ecological hazards connected with chelate-assisted phytoextraction, as phytotoxicity or metal leaching. The amendment of soil with some organic compounds was found to be effective at enhancing phytoremediation and biodegradation of co-contaminated soils in pot experiments in which *Alyssum lesbiacum* was grown in nickel and PAH spiked-soil (Singer et al. 2007). In that study, treatment with a combination of a surfactant (sorbitan trioleate), a PAH biodegradation inducer (salicylic acid) and a Ni-chelator (histidine) induced high biomass production by *Alyssum lesbiacum*.

Finally, rhizobacteria can be stimulated by the addition of agrowaste residues (Azcon et al. 2009) or chelates to ameliorate plant growth and metal phytoextraction. For example, Chen et al. (2006) found that microbial communities of *Elsholtzia splendens* and *Trifolium repens* grown on Cu contaminated soil amended with glucose and citric acid facilitated Cu solubilisation without inhibiting the microbial community.

9 Conclusions

Rhizoremediation and phytoextraction might be effective approaches to the remediation of soils contaminated by metals and organics. The exploitation of symbiotic relationships between plants and rhizobacteria should lead to better clean-up of polluted soils. However, the complexity and heterogeneity of co-contaminated soils require integrated approaches of the rhizosphere management. In particular, concerted efforts should be focused on the development of suitable environmental and agricultural engineering techniques that will have a major impact on the efficiency of plant cultivation. The selection of more efficient plant varieties and soil amendments and the optimization of agronomic practices should provide improved phytoremediation. The combined use of phytoextraction and rhizodegradation crops, the inoculation of roots or seeds of hyperaccumulator plants, the genetic manipulation of hyperaccumulators expressing ACC deaminase and other specific organic-degradative genes may be a breakthrough in the enhanced removal of heavy metals and organics from the soil environment.

Acknowledgments This work was supported by PUR 2008 from the University of Milan, Italy.

References

Andreoni V, Gianfreda L (2009) PAH bioremediation by microbial communities and enzymatic activities. In. RH Crabtree (ed) Handbook of green chemistry, vol 3. Biocatalysis, Wiley-VCH, Germany, pp 243–268

Angle JS, Chaney RL (1989) Cadmium resistance screening in nitriloacetetate-buffered mineral media. Appl Environ Microbiol 55:2101–2114

Azcon R, Medina A, Roldan A, Biro B, Vivas A (2009) Significance of treated agrowaste residue and autochthonous inoculates (arbuscular mycorrhizal fungi and *Bacillus cereus*) on bacterial community structure and phytoextraction to remediate soils contaminated with heavy metals. Chemosphere 75:327–334, doi:10.1016/j.chemosphere.2008.12.029

Babalola OO, Osir EO, Sanni AI, Odhiambo GD, Bullimo WD (2003) Amplification of 1-amino-cyclopropane -1-carboxylic (ACC) deaminase from plant growth promoting rhizobacteria in Striga-infested soil. African J Biotechnol 2:157–160

Barkay T, Miller SM, Summers AO (2003) Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol Rev 27:355–384

Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S (2005) Cadmium-tolerant plant growth promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). Soil Biol Biochem 37:241–250, doi:10.1016/j.soilbio.2004.07.033

Bizily S, Kim T, Kandasamy MK, Meagher RB (2003) Subcellular targeting of methylmercury lyase enhances its specific activity for organic mercury detoxification in plants. Plant Physiol 131:463–471

Bizily S, Rugh CL, Summers AO, Meagher RB (1999) Phytoremediation of methylmercury pollution: MerB expression in *Arabidopsis thaliana* confers resistance to organomercurials. Proc Natl Acad Sci U S A 96:6808–6813

Blaha D, Prigent-Combaret C, Mirza MS, Moenne-Loccoz Y (2006) Phylogeny of the 1-aminocyclopropane-1-carboxylic acid deaminase- encoding gene *acdS* in phytobeneficial and pathogenic Proteobacteria and relation with strain biogeography. FEMS Microbiol Ecol 56:455–470

Bloemberg GV, Wijfjes AHM, Lamers GEM, Stuurman N, Lugtenberg BJJ (2000) Simultaneous imaging of *Pseudomonas fluorescens* WCS365 populations expressing three different autofluorescent proteins in the rhizosphere: New perspectives for studying microbial communities. Mol Plant-Microbe Interact 13:1170–1176

Boldt TS, Sorensen J, Karlson U, Molin S, Ramos C (2004) Combined use of different gfp reporters for monitoring single-cell activities of a genetically modified PCB degrader in the rhizosphere of alfalfa. FEMS Microbiol Ecol 48:139–148

Broek AV, Venderleyden J (1995) The role of bacterial motility, chemotaxis, and attachment in bacterial-plant interactions. Mol Plant Microbe Interact 8:800–810

Burd D, Dixon G, Glick RB (1998) A plant growth promoting bacterium that decreases nickel toxicity in seedlings. Appl Environ Microbiol 64:3663–3668

Butler JL, Williams MA, Bottomley PJ, Myrold DD (2003) Microbial community dynamics associated with rhizosphere carbon flow. Appl Environ Microbiol 69:6793–6800

Cattani I, Fragoulis G, Boccelli R, Capri E (2006) Copper bioavailability in the rhizosphere of maize (*Zea mays* L.) grown in two Italian soils. Chemosphere 64:1972–1979, doi: 10.1016/j.chemosphere.2006.01.007

Cavalca L, Dell'Amico E, Andreoni V (2004) Intrinsic bioremediability of an aromatic hydrocarbon-polluted groundwater: diversity of bacterial population and toluene monooxygenase genes. Appl Microbiol Biotechnol. 64:576–587

Chaudhry Q, Blom-Zandstra M, GuptaS, Joner EJ (2005) Utilizing the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. Environ Sci Poll Res 12:34–48

Chen Y, Wang Y, Wu W, Lin Q, Xue S (2006) Impacts of chelate-assisted phytoremediation on microbial community composition in the rhizosphere of a copper accumulator and non-accumulator. Sci Total Environ 356:247–255

Cottrell MT, Kirchman DL (2000) Natural assemblage of marine proteobacteria and members of the Cytofaga-Flavobacter cluster consuming low- and high-molecular–weight dissolved organic matter. Appl Environ Microbiol 66:1692–1697

Cui YS, Dong YT, Li HF, Wang QR (2004) Effect of elemental sulphur on solubility of soil heavy metals and their uptake by maize. Environ Int 30(3):323–328, doi: 10.1016/S0160-4120(03)00182-X

Cunningham SD, Ow DW (1996) Promises and prospects of phytoremediation. Plant Physiol 110:715–719

Daane LL, Harjonio I, Zylstra GJ, Hagblom MM (2001) Isolation and characterization of polycyclic aromatic hydrocarbon-degrading bacteria associated with the rhizosphere of salt marsh plants. Appl Environ Microbiol 67:683–2691

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

Doelman P, Jansen E, Michels M, VanTil M (1994) Effects of heavy metals in soil on microbial diversity and activity as shown by the sensitivity-resistance index, an ecologically relevant parameter. Biol Fert Soils 17:177–184

Espinosa-Urgel M, Kolter R, Ramos JL (2002) Root colonization by *Pseudomonas putida*: Love at first sight. Microbiology 143:341–343

Farwell AJ, Vesely S, Nero V, Rodriguez H, McCormack K (2006) The use of transgenic canola (*Brassica napus*) and plant growth-promoting bacteria to enhance plant biomass at a nickel-contaminated field site. Plant Soil 288:309–318

Gerhardt KE, Greenberg DM, Glick BR (2006) The role of ACC deaminase in facilitating phytoremediation of organics, metals and salts. Curr Tren Microbiol 2:61–72

Gianfreda L, Rao MA (2004) Potential of extracellular enzymes in remediation of polluted soils: A review. Enzyme Microbial Technol 35:339–354

Gibson TG, Parales ER (2000) Aromatic hydrocarbon dioxygenases in environmental biotechnology. Current Opin Biotechnol 11:26–243

Glick BR (2003) Phytoremediation: Synergistic use of plants and bacteria to clean up the environment. Biotechnol Advan 21:83–393

Glick BR, Cheng Z, Czarny J, Duan J (2007) Promotion of plant growth by ACC deaminase–producing soil bacteria. European J Plant Pathol 119:329–339

Gomes NCM (2003) Dynamics of fungal communities in bulk and maize rhizosphere soil in the tropics. Appl Environ Microbiol 69:3758–3766

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: Communalities and distinctions in the plant-bacterium signalling processes. Soil Biol Biochem 37:395–412

Grichko VP, Glick RB (2001) Amelioration of flooding stress by ACC deaminase containing plant growth-promoting bacteria. Plant Physiol Biochem 9:11–17, doi:10.1016/S0981-9428(00)01212-2

Grichko VP, Filby B, Glick RB (2000) Increased ability of transgenic plants expressing the bacterial enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, Pb, and Zn. J Biotechnol 81:45–45, doi:10.1016/S0168-1656(00)00270-4

Griffiths RI, Whiteley AS, O'Donnell AG, Bailey JM (2003) Physiological and community responses of established grassland bacterial populations to water stress. Appl Environ Microbiol 69:6961–6968, doi:10.1128/AEM.69.12.6961–6968

Hinsinger P, Plassard C, Tang C, Jaillard B (2003) Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant Soil 248:43–59, doi: 10.1023/A:1022371130939

Hontzeas N, Zoidakis J, Glick BR, Abu-Omar MM (2004) Expression and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the rhizobacterium *Pseudomonas putida* UW4: A key enzyme in bacterial plant growth promotion. Biochem Biophy Acta 1073:11–19

Huang XD, El-Alawi Y, Penrose DM, Glick BR, Greenberg BM (2004) A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environ Poll 130:465–476

Hughes MN, Poole RK (1989) Metal toxicity. In: Hughes MN, Poole RK (eds) Metals and microorganisms. Chapman and Hall, New York, pp 252–302

Jankong P, Visoottiviseth P, Khokiattiwong S (2007) Enhanced phytoremediation of arsenic contaminated land. Chemosphere 68(10):1906–1912, doi:10.1016/j.chemosphere.2007.02.061

Janssen DB, Dinkla IJ, Poelarends GJ, Terpstra P (2005) Bacterial degradation of xenobiotic compounds: evolution and distribution of novel enzyme activities. Environ Microbiol 7:1868–1882

Jansson JK (2003) Marker and reporter genes: Illuminating tools for environmental microbiologists. Curr Opinion Microbiol 6:310–316

Kabata-Pendias A (2004) Soil-plant transfer of trace elements - an environmental issue. Geoderma 122:143–149.

Kayser A, Schroder TJ, Grunwald A, Schulin R (2001) Solubilization and plant uptake of zinc and cadmium from soils treated with elemental sulphur. Int J Phytorem 3:381–400

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

Krämer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature 379:635–638, doi:10.1038

Kuo CW, Genthner BRS (1996) Effect of added heavy metal ions on biotransformation and biodegradation of 2-chlorophenol and 3-chlorobenzoate in anaerobic bacterial consortium. Appl Environ Microbiol 62:2317–2323

Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31(1):109–120

Lee W, Wood TK, Chen W (2006) Engineering TCE-degrading rhizobacteria for heavy metal accumulation and enhanced TCE degradation. Biotechnol Bioengineer 95:399–403

Leigh MB, ProuzováP, Macková M, Macek T, Nagle DP, Fletcher JS (2006) Polychlorinated biphenyl (PCB)-degrading bacteria associated with trees in a PCB-contaminated site. Appl Environ Microbiol 72:2331–2342

Liste HH, Alexander M (2000) Accumulation of phenanthrene and pyrene in rhizosphere soil. Chemosphere 40:11–14

Liu L, Jiang CY, Liu XY, Wu JF, Han JG, Liu SJ (2007) Plant–microbe association for rhizoremediation of chloronitroaromatic pollutants with *Comamonas* sp. strain CNB-1. Environ Microbiol 9:465–473

Loosemore N, Straczek A, Hinsinger P, Jaillard B (2004) Zinc mobilisation from a contaminated soil by three genotypes of tobacco as affected by soil and rhizosphere pH. Plant Soil 260 (1–2):19–32

Luo YM, Christie P, Baker AJM (2000) Soil solution Zn and pH dynamics in non-rhizosphere soil and in the rhizosphere of *Thlaspi caerulescens* grown in a Zn/Cd-contaminated soil. Chemosphere 41(1/2):161–164, doi:10.1016/S0045-6535(99)00405-1

Ma W, Sebastianova S, Sebestian J, Burd GI, Guinel F, Glick BR (2003) Prevalence of 1-aminocyclopropane-1-carboxylate deaminase in Rhizobia spp. Antonie van Leeuwenhoek 83: 285–291

Macek T, Mackova M, Kas J (2000) Exploitation of plants for the removal of organics in environmental remediation. Biotechnol Adv 18:23–24, doi:10.1016/S0734-9750(99)00034-8

Macur RE, Jackson CR, Botero LM, McDermott DR, Inskeep WP (2004) Bacterial populations associated with the oxidation and reduction of arsenic in an unsaturated soil. Environ Sci Technol 38:104–111

Madhaiyan M, Kim BY, Poonguzhali S, Kwon SW, Song MH, Ryu JH, Go SJ, Koo BS, Sa TM (2007) *Methylobacterium oryzae* sp. nov., an aerobic, pink-pigmented, facultatively methylotrophic, 1-aminocyclopropane-1-carboxylate deaminase-producing bacterium isolated from rice. Int J Syst Evol Microbiol 57:326–331

Mark GL, Dow JM, Kiely PD, Higgins H, Haynes J, Baysse C (2005) Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe-plant interactions. Proc Natl Acad Sci U S A 102:17454–17459.

Mars AE, Houwing J, Dolfing J Janssen DB (1996) Degradation of toluene and trichloroethylene by *Burkholderia cepacia* G4 in growth-limited fed-batch culture. Appl Environ Microbiol 62:886–891.

Mayak S, Tirosh T, Glick BR (2004) Plant-growth promoting bacteria that confer rsistance to water stress in tomatoes and peppers. Plant Sci 166:525–530

Meagher RB, Heaton ACP (2005) Strategies for the engineered phytoremediation of toxic element pollution. J Ind Microbiol Biotechnol 32:502–513

Messens J, Hayburn G, Desmyter A, Laus G, Wyns L (1999) The essential catalytic redox couple in arsenate reductase from *Staphylococcus aureus*. Biochemistry 38:16858–16865

Newman LA, Reynolds CM (2004) Phytodegradation of organic compounds. Curr Opin Biotechnol 15:225–230

Nicol GW, Glover LA, Prosser JI (2003) Spatial analysis of archea community structure in grassland soil. Appl Environ Microbiol 69:7420–7429

Nie L, Shah S, Rashid A, Burd GI, Dixon G, 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

Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbil Rev 27:313–339

Palmroth MRT, Koskinen PEP, Pichtel J, Vaajasaari K, Joutti A, Tuhkanen TA, Puhakka JA (2006) Field scale assessment of phytotreatment of soil contaminated with weathered hydrocarbons and heavy metals. J Soils Sed 6:128–136

Pernthaler A, Amann R (2004) Simultaneous fluorescence in situ hybridization of mRNA and rRNA in environmental bacteria. Appl Environ Microbiol 70:5426–5433

Phale PS, Basu A, Majhi PD, Deveryshetty J, Vamsee-Krishna C, Shrivastava R (2007) Metabolic diversity in bacterial degradation of aromatic compounds. OMICS A J Integ Biol 11(3): 252–279

Pilon-Smits E (2005) Phytoremediation. Ann Rev Plant Biol 56:15–39, doi:10.1146/annurev.arplant.56.032604.144214

Ramamoorthy V, Viswanathan R, Raguchander T, Prakasam V, Smaiyappan R (2001) Induction of systemic resistance by plant growth-promoting rhizobacteria in crop plants against pests and diseases. Crop Protec 20:1–11

Rangel-Castro JI, Killham K, Ostle N, Nicol GW, Anderson IC, Scrimgeour CM (2005) Stable isotope probing analysis of the influence of liming on root exudates utilization by soil microorganisms. Environ Microbiol 7:828–838, doi:10.1111/j.1462-2920.2005.00756.x

Ranjard L, Brothier E, Nazaret S (2000) Sequencing bands of ribosomal intergenic spacer analysis fingerprints for characterization and microscale distribution of soil bacterium populations responding to mercury spiking. Appl Environ Microbiol 66(12):5334–5339

Reed MLE, Glick BR (2005) Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Canadian J Microbiol 51:1061–1069

Roane TM, Josephson KL, Pepper IL (2001) Dual-bioaugmentation strategy to enhance remediation of co-contaminated soil. Appl Environ Microbiol 67:3208–3215

Roane TM, Pepper IL (1997) Microbial remediation of soils co-contaminated with 2,4-dichlorophenoxy acetic acid and cadmium. In: 12th annual conference on hazardous waste research: building partnerships for innovative technologies, 19–22 May 1997, Kansas City, Missouri

Rosen BP (2002) Biochemistry of arsenic detoxification. FEBS Lett 529:86–92

Ruggiero P, Pizzigallo MDR, Crecchio C (2002) Effects of soil abiotic processes on the bioavailability of anthropogenic organic residues. In: Violante A, Huang PM, Bollag JM, Gianfreda L (eds) Development in soil science, vol 28B. Elsevier, Amsterdam, pp 95–133

Rugh CL, Wilde D, Stack NM, Thompson DM, Summers AO, Meagher RB (1996) Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial *merA* gene. Proc Natl Acad Sci U S A 93:3182–31087

Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Ensley BD, Chet I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. BioTechnology 13:468–474

Saltikov CW, Newman DK (2003) Genetic identification of a respiratory arsenate reductase. Proceed Nati Acad Sci USA 10(16):10983–10988

Sandrin TR, Maier RM (2003) Impact of metals on the biodegradation of organic pollutants. Environ Perspec 111:1093–1100

Sanseverino J, Werner C, Fleming J, Applegate B, King JM, Sailer GS (1993–1994) Molecular diagnostic of polycyclic aromatic hydrocarbon biodegradation in manufactured gas plant soils. Biodegradation 4:303–321

Saravanakumar D, Samyappan R (2007) ACC deaminase from Pseudomonas fluorescens mediated saline resistance in groundnut (*Arachis hypogea*) plants. J Appl Microbiol 102: 1283–1292

Schmidt U (2003) Enhancing phytoextraction: The effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. J Environ Qual 32: 1939–1954

Schwab AP, Al-Assi AA, Banks MK (1998) Adsorption of naphthalene onto plant roots. J Environ Qual 27:220–224

Shah S, Li J, Moffatt BA, Glick BR (1998) Isolation and characterization of ACC deaminase genes from two different plant-growth promoting rhizobacteria. Can J Microbiol 44:833–843

Shen ZG, Liu YL (1998) Progress in the study on plants that hyperaccumulate heavy metals. Plant Physiol Composit 34:133–139

Shi W, Becker J, Bischoff M, Turco RF, Konopka AE (2002) Association of microbial community composition and activity with lead, chromium, and hydrocarbon contamination. Appl Environ Microbiol 68:3859–3866

Shim H, Chauhan S, Ryoo D, Bowers K, Thomas SM, Canada KA, Burken JG, Wood TK (2000) Rhizosphere competitiveness of trichloroethylene degrading, poplar-colonizing recombinant bacteria. Appl Environ Microbiol 66:4673–4678

Siciliano SD, Fortin N, Mihoc A, Wisse G, Labelle S, Beaumier D (2001) Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. Appl Environ Microbiol 67:2469–2475, doi: 10.1128/AEM.67.6.2469-2475.2001

Silva-Gonzaga MI, Santos JAG, Ma LQ (2006) Arsenic chemistry in the rhizosphere of *Pteris vittata* L. and *Nephrolepis exaltata* L. Environ Poll 143:254–260

Silver S, Phung LT (2005) Genes and enzymes involved in the bacterial oxidation and reduction of inorganic arsenic. Appl Environ Microbiol 71:599–608

Singer AC, Bell T, Heywood CA, Smith JAC, Thompson IP (2007) Phytoremediation of mixed-contaminated soil using the hyperaccumulator plant *Alyssum lesbiacum*: Evidence of histidine as a measure of phytoextractable nickel. Environ Poll 147:74–82, doi: 10.1016/j.envpol.2006.08.029

Singer AC, Thomson LP, Bajley MJ (2004) The tritrophic trinity: A source of pollutant-degrading enzymes and its implication for phytoremediation. Curr Opinion Microbiol 7:239–244

Smith KE, Schwab AP, Banks MK (2007) Phytoremediation of polychlorinated biphenyl (PCB)-contaminated sediment: a greenhouse feasibility. J Environ Qual 36:239–244, doi: 10.2134/jeq2006.0089

Soderberg KH, Probanza A, Jumpponen A, Baath E (2004) The microbial community in the rhizosphere determined by community level-phisyological profile (CCLP) and direct soil- and cfu-PLFA techniques. Appl Soil Ecol 25:135–145

Sokhn J, De Leij FAMM, Hart TD, Lynch JM (2001) Effect of copper on the degradation of phenanthrene by soil microorganisms. Lett Appl Microbiol 33:164–168

Spiegelman D, Whissell G, Greer CW (2005) A survey of the methods for the characterization of microbial consortia and communities. Canadian J Microbiol 51355–386

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

Thompson OA, Wolf DC, Mattice JD, Thoma GJ (2008) Influence of nitrogen addition and plant root parameters on phytoremediation of pyrene-contaminated soil. Water Air Soil Poll 189: 37–47.

Tom-Peterson A, Hosbond C, Nybroe O (2001) Identification of copper-induced genes in *Pseudomonas fluorescens* and use of a reporter strain to monitor bioavailable copper in soil. FEMS Microbiol Ecol 38:59–67

Wang L, Knill E, Glick BR, Defago G (2000) Effects of transferring 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase genes into *Pseudomonas fluorescens* strain CHA0 and its *gacA* derivative CHA96 on their growth-promoting and disease-suppressive capacities. Canadian J Microbiol 46:898–907

Wenzel WW, Bunkowski M, Puschenreiter M, Horak O (2003) Rhizosphere characteristics of indigenously growing nickel hyperaccumulator and excluder plants on serpentine soil. Environ Poll 123(1):131–138, doi: 10.1016/S0269-7491(02)00341-X

Whiting SN, Leake JR, McGrath SP, Baker AJM (2001) Assessment of Zn mobilization in the rhizosphere of *Thlaspi caerulescens* by bioassay with non-accumulator plants and soil extraction. Plant Soil 237(1):147–156, doi: 10.1023/A:1013365617841

Wong KW, Toh BA, Ting YP, Obbard JP (2005) Biodegradation of phenantherene by the indigenous microbial biomass in a zinc amended soil. Lett Appl Microbiol 40:50–55

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

Xie HL, Jiang RF, Zhang FS, McGrath SP, Zhao FJ (2009) Effect of nitrogen form on the rhizosphere dynamics and uptake of cadmium and zinc by the hyperaccumulator *Thlaspi caerulescens*. Plant Soil 318:205–215, doi: 10.1007/s11104-008-9830-y

Yateem A, Al-Sharrah T, Bin-Haji A (2007) Investigation of microbes in the rhizosphere of selected grasses for rhizoremediation of hydrocarbon-contaminated soils. Soil Sedim Contam 16:269–280, doi: 10.1080/15226510802096143

Zaccheo P, Crippa L, Di Muzio Pasta V (2006) Ammonium nutrition as a strategy for cadmium mobilisation in the rhizosphere of sunflower. Plant Soil 283:43–56, doi: 10.1007/s11104-005-4791-x

Zehnder GW, Murphy JF, Sikora EJ, Kloepper JW (2001) Application of rhizobacteria for induced resistance. European J Plant Pathol 107(1):39–50

Zhao FJ, Hamon RE, McLaughlin MJ (2001) Root exudates of the hyperaccumulator *Thlaspi caerulescens* do not enhance metal mobilization. New Phytol 151(3):613–620, doi: 10.1046/j.0028-646x.2001.00213.x

Zhou J (2003) Microarrays for bacterial detection and microbial community analysis. Curr Opinion Microbiol 6:1–7