Minireview

Bacterial responses and interactions with plants during rhizoremediation

Ana Segura,[1]* Sara Rodríguez-Conde,[1] Cayo Ramos[2] and Juan L. Ramos[1]

[1]*Consejo Superior de Investigaciones Científicas, Estación Experimental del Zaidín, Department of Environmental Microbiology, Professor Albareda 1, E-18008 Granada, Spain.*
[2]*University of Malaga, Faculty of Sciences, Section of Genetics, Campus de Teatinos s/n, E-29071 Malaga, Spain.*

Summary

With the increase in quality of life standards and the awareness of environmental issues, the remediation of polluted sites has become a priority for society. Because of the high economic cost of physico-chemical strategies for remediation, the use of biological tools for cleaning-up contaminated sites is a very attractive option. Rhizoremediation, the use of rhizospheric microorganisms in the bioremediation of contaminants, is the biotechnological approach that we explore in this minireview. We focus our attention on bacterial interactions with the plant surface, responses towards root exudates, and how plants and microbes communicate. We analyse certain strategies that may improve rhizoremediation, including the utilization of endophytes, and finally we discuss several rhizoremediation strategies that have opened ways to improve biodegradation.

Introduction

Microbial–plant interactions were extensively studied during the second half of the last century; however, these studies focused mainly on plant–pathogen interactions or the well-known plant–saprophytic interactions. In the last decade, the ecology of microbes in the rhizosphere, defined by Hiltner (1904) as the area influenced by the root system, has provided new insights in microbial com-

munication and their dialogue with plants (Kiely *et al.*, 2006; Shaw *et al.*, 2006; Danhorn and Fuqua, 2007). It has been well documented that rhizospheric microorganisms can promote plant growth by many different mechanisms, including nitrogen fixation, nutrient mobilization (i.e. phosphorous), or even by the production of plant growth regulators. Beneficial microbial interactions also include the inhibition of pathogen growth by nutrient competition, as well as the production of antibiotics and toxins. Furthermore, certain non-pathogenic bacteria can induce plant defence mechanisms (Handelsman and Stabb, 1996; Sticher *et al.*, 1997; Bender *et al.*, 1999; Lugtenberg *et al.*, 2002; Haas and Défago, 2005; Morgan *et al.*, 2005; Tian *et al.*, 2007).

The classical enrichment culture techniques, together with new '-omics' technologies, have been used to demonstrate that the number of microbes in the rhizosphere is larger than in the bulk soil and that they are also metabolically more active (Campbell and Greaves, 1990; Ramos *et al.*, 2000a,b; Kent and Triplett, 2002). This is the so-called rhizosphere effect, which consists of the plant excreting a number of compounds that can be used as carbon, nitrogen, sulfur, or phosphorous sources by microbes to proliferate and reach high cell densities in the area surrounding the plant's root (Rovira, 1965; Merckx *et al.*, 1986; Smalla *et al.*, 2001; Walker *et al.*, 2003; Morgan *et al.*, 2005). Plant roots provide a large surface on which microbes can proliferate, can be transported through the soil in terms of both spreading and depth and, as mentioned above, the root provides nutrients and through its soil penetration, facilitates oxygen exchange allowing the proliferation of aerobic microorganisms. In addition, root exudates contain different phenolic compounds, which can act as inducers of different contaminant catabolic pathways (Fletcher and Hedge, 1995; Shurtliff *et al.*, 1996). Despite the general rhizosphere effect, an increasing number of reports have indicated that the bacterial composition in the rhizosphere is affected by complex interactions, including soil type, plant species and root zone localization (Marschner *et al.*, 2001; Chen *et al.*, 2006).

In this minireview, we focus on the behaviour of bacteria with bioremediation potential in the roots of plants.

Although fungi also have the potential to proliferate and remove pollutants present in the rhizosphere, they are not the subject of this minireview.

Advantages of phytorhizoremediation

Remediation options using physico-chemical treatments are expensive and, in general, are environmentally invasive. Their high cost sometimes makes them prohibitive, especially for the treatment of large areas with medium/ low levels of contamination (Cunningham and Ow, 1996). In these cases, biological treatments are a good alternative (Cunningham *et al.*, 1996; Doty, 2008). The biological treatments used in recent years have had different degrees of success. *On-site* techniques, such as land-farming or composting, are promising options, but involve manipulating soils and sometimes provoke the mobilization of the contaminant. *In situ* techniques, such as the inoculation of microorganisms with appropriate catalytic properties, bioaugmentation, and soil fertilization, are costly and sometimes unsuccessful (Colleran, 1997).

Despite the fact that there are an impressive number of publications reporting the isolation of microbes with the capacity to degrade contaminants (Cerniglia, 1993; Urbance *et al.*, 2003; Parales and Haddock, 2004), most attempts to re-introduce these microorganisms into soils to remove pollutants have been unsuccessful. This is probably due to the lack of knowledge regarding the behaviour of these microbes in the environment. Factors, such as soil type, soil moisture, temperature, limitations in microbial reactions to environmental stress conditions (i.e. the toxicity of the contaminant and the scarcity of nutrients), predators, and the inability of inoculated microbes to compete with autochthonous microflora, have been reported to influence the performance of microbes during bioremediation (Goldstein *et al.*, 1985; van Veen *et al.*, 1997; Head, 1998).

As an alternative to the failures in the field of bioaugmentation, phytoremediation has been proposed as an attractive strategy to achieve the efficient removal of pollutants. Plants are easy to monitor, they can be used to eliminate a wide range of pollutants, and agriculture techniques are available to minimize the costs of the treatment. Phytoremediation strategies include: phytostabilization, where plants, either physically or by the action of the root exudates, help sequester the contaminant in the soil making it less bioavailable; phytovolatilization, where the plants take up the contaminant from the soil and transform it into a volatile compound that is released into the atmosphere for dispersal; phytoextraction, which involves the accumulation of toxic compounds in the harvestable part of a plant; rhizoremediation, involving the elimination of the contaminant by the microbes in the rhizosphere; and phytoremediation, a term which refers to

the transformation of the contaminant by the plant metabolism (Cunningham *et al.*, 1996; Salt *et al.*, 1998; Susarla *et al.*, 2002). *A priori*, the easiest way to design a phytoremediation protocol would be to use a single 'degradative organism'; unfortunately, plants, in general, do not mineralize contaminants, so their potential use in phytoremediation is limited. Therefore, a combination of plants and microbes seems to be a better approach.

Although phytoremediation is a promising option, it also has drawbacks. Pollutants above a certain level can be toxic to both the plants and the associated microorganisms (van Dillewijn *et al.*, 2008), plant metabolism can transform the contaminant (at least temporarily) into a more toxic chemical (Trenck and Sandermann, 1980; Hughes *et al.*, 1997) or the plant can mobilize the contaminant from the soil to an aerial part where it can be introduced into the food chain.

Transgenic plants with enhanced potential for phytoremediation have been constructed. These transgenic plants have been provided with eukaryotic (i.e. cytochrome P450 monooxygenases, glutathione S-transferases and metallothionein) or bacterial genes (i.e. pentaerythritol tetranitrate redutase, mercuric ion reductase, and organomercurial lyase) and they represent good alternatives for phytoremediation. However, the release of genetically modified organisms still has legal restrictions in many countries, which is a drawback for the use of transgenic plants. The use of transgenic plants for phytoremediation was recently reviewed (Doty, 2008; Van Aken, 2008) and will not be discussed further in this article.

Life in the rhizosphere

To design a successful rhizoremediation strategy there are at least two basic requirements that should be fulfilled. First, microbes must be able to proliferate in the root system, a process which multiplies their catalytic potential (Salt *et al.*, 1998). Second, catabolic pathways must be operative (Böltner *et al.*, 2008).

Root colonization

Bacterial attachment to plant roots is an early step in plant root colonization. Initial approaches for identifying and studying genes involved in root colonization were based on the use of random or directed mutagenesis to isolate mutants impaired for colonization. Bacterial attachment has been extensively studied in rhizobacteria and although the molecular basis is still not completely understood, the general mechanism seems to be mediated by surface proteins, capsular polysaccharides, flagella and chemotaxis (de Weger *et al.*, 1987; Broek *et al.*, 1998; Dekkers *et al.*, 1998a; Palumbo *et al.*, 1998; de Weert

et al., 2002; Capdevila *et al.*, 2004; Rodríguez-Navarro *et al.*, 2007). Interestingly, the rhizosphere can select mutants in which functions, such as motility, are improved (Sánchez-Contreras *et al.*, 2002; Martínez-Granero *et al.*, 2006). Functions involved in root colonization are often evident in competition experiments where colonization of the wild-type versus mutants is assayed. This can be linked to general physiological deficiencies due to defects in the synthesis of amino acids and vitamin B1, the ability to grow on organic acids, or mutations in respiratory chain genes (reviewed by Lugtenberg and Dekkers, 1999). Others functions that were previously determined to be specific of certain bacteria (i.e. efflux pump genes in *Agrobacterium*) (Palumbo *et al.*, 1998), have now been revealed to be widespread mechanisms for survival in the rhizosphere (see below).

In addition to characterize mutants that are deficient in root colonization, the identification of genes that are specifically induced in the presence of roots or root exudates allows for the investigation of the specific gene expression programme that microbes must establish to proliferate at the plant root. The first global approach was based on 'in vivo expression technology', which involved selecting microbial promoters active in the rhizosphere (Rainey and Preston, 2000; Rediers *et al.*, 2005). This approach enabled the identification of 20 genes in the saprophytic bacteria *P. fluorescens* (Rainey, 1999), 28 genes in the biodegradative *P. putida* KT2440 (Ramos-González *et al.*, 2005), and 29 genes in the nodulating bacteria *Rhizobium leguminosarum* (Barr *et al.*, 2008); all of these genes were induced during rhizosphere colonization. Rhizosphere-induced fusions in the three bacteria included genes with probable functions in the cell envelope, chemotaxis, motility, transport, secretion, DNA metabolism, stress mechanisms, regulation, energy metabolism, detoxification and protein synthesis. Some of these functions, including the ColR/ColS two-component system, the flagellar genes and efflux pumps, have been implicated in root colonization by independent laboratories using the mutant analysis approach (Dekkers *et al.*, 1998b; Palumbo *et al.*, 1998; Lugtenberg and Dekkers, 1999; Capdevila *et al.*, 2004). With the advent of micro-array technology, more global approaches are being considered (Kiely *et al.*, 2006). Three recent papers (Mark *et al.*, 2005; Matilla *et al.*, 2007; Attila *et al.*, 2008) have revealed that nearly 200 promoters in different strains of *Pseudomonas* are specifically induced in the presence of roots exudates or plant roots (highlighted by van Dillewijn, 2008). The results of these transcriptional analysis experiments confirmed the role of certain genes, such as those involved in flagella and vitamin B1 biosynthesis, in root colonization and also the involvement of genes related to specific nutrient acquisition, the adaptation to adverse conditions, the efflux of toxic compounds, and many regulatory pro-

teins. The authors also identified genes that were specifically induced using the system under study. Although these studies have been done using simple models (one plant and one bacteria), overall, they reveal that bacterial fitness in the rhizosphere is a complex phenotype that is affected by many different traits and environmental factors.

Successful rhizosphere colonization depends not only on interactions between the plants and the microorganisms of interest, but also on interactions with other rhizospheric microorganisms and the environment. Molecular techniques, such as denaturing or temperature gradient gel electrophoresis have allowed researchers to follow the modifications in bacterial communities after environmental perturbations, including the introduction of plants or biodegradative bacteria, changes in temperature, or the addition of contaminants (Smit *et al.*, 2001; Kent and Triplett, 2002; de Cárcer *et al.*, 2007; Kielak *et al.*, 2008). Several techniques to follow seed and root colonization by bacteria have been developed during the last 15 years, which mainly include *in situ* hybridization assays using fluorescent probes and the visualization of bacteria that carry the *luxAB* genes encoding bacterial luciferase (Fig. 1), the green fluorescent protein, or another reporter gene (Tombolini *et al.*, 1999; Broek *et al.*, 1998; Ramos *et al.*, 2000a,b; 2001). These techniques have been used to illustrate that introduced microorganisms are often unable to compete with indigenous microorganisms or are unable to establish high numbers in the rhizosphere (Rattray *et al.*, 1995; Lübeck *et al.*, 2000). Some bacteria have developed strategies to out-compete other microorganisms by delivering toxins, using extremely efficient nutrient utilization systems, or by physical exclusion (Lugtenberg *et al.*, 1991). However, many other factors involved in successful colonization, under non-sterile conditions, remain unknown.

Mounting evidence indicates that plants are able to select the bacteria living in their rhizosphere by different mechanisms, including root architecture, the modification of soil conditions, or the exudation of specific compounds. Each plant exudes specific compounds, which are dependent on the plant's particular secondary metabolism. Some plants can promote the growth of bacteria that are able to degrade certain compounds, while others secrete toxic compounds that select for tolerant bacteria, and some plants are able to secrete hydrolases that degrade acyl homoserine lactones, thus inhibiting bacterial quorum sensing (reviewed by Hartmann *et al.*, 2009).

We can conclude that the rhizosphere is a highly dynamic environment, where root exudates, soil temperature, humidity and other factors are constantly changing. Moreover, bacteria are sending and receiving signals from plants, other bacteria, and from the environment. In this environment, bacteria are competing for limited nutrients
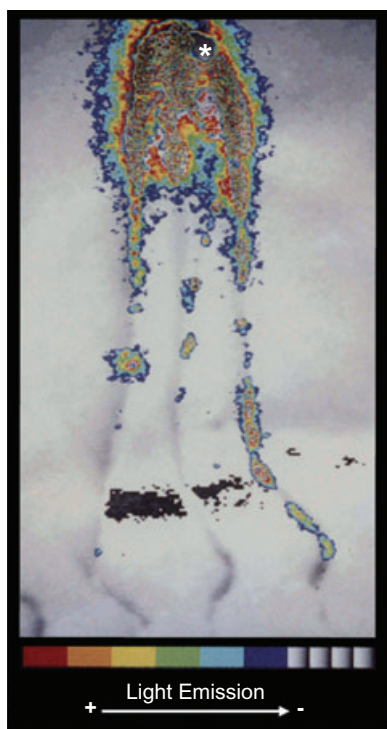
Fig. 1. Light emission of a *luxAB*-tagged *P. putida* KT2440 derivative (*P. putida* strain S1B1) colonizing the root system of *Zea mays*. A sterile maize seed (*) was coated with mid-log phase grown cells of this strain and germinated in vermiculite. Bioluminescence in the root system of the developing maize plant was detected 15 days after planting by photon counting using a CCD camera. Dark-field-exposure (30 s) was processed with Adobe Photoshop software. The relative intensity of light emission is indicated by the colour scale.

and are exposed to relatively high levels of putative toxic compounds from plant exudates, from other rhizospheric microorganisms, and, if living in contaminated soils, from the toxicity of the contaminant.

The expression of catabolic genes in the rhizosphere

The list of contaminant-degrading bacteria associated with plant rhizospheres is very extensive. In a recent survey done in our laboratory, several rhizosphere-isolated bacteria, belonging to the genera *Arthrobacter*, *Burkholderia*, *Mycobacterium*, *Novosphigobium*, *Pseudomonas* and *Sphingomonas*, have been characterized by their ability to degrade phenanthrene (S. Rodríguez-Conde and A. Segura, unpublished). Many other rhizospheric bacteria have previously been described as able to degrade a wide variety of contaminants (Daane *et al.*, 2001; Kuiper *et al.*, 2001; Jussila *et al.*, 2006, among others). For the efficient removal of soil contaminants, not only do microbes with the appropriate catabolic genes have to be maintained in the rhizosphere, but the genes have to be conveniently expressed and be free of the

catabolite repression effect, in which microbes use a given carbon or nitrogen source preferentially over others (Burken, 2004). The easiest assays to probe for microbial activity against pollutants are those where it is possible to monitor CO_2 evolution when the chemical under scrutiny is available as a labelled compound (Parkin and Shelton, 1992; Levanon, 1993; Bending *et al.*, 2001; Rasmussen *et al.*, 2004). More sophisticated experiments can be done if substrates are labelled with a stable isotope to study the incorporation of a heavier C- or N-source into cell components (Madsen, 2006). The utilization of reporter genes to study the expression of catabolic genes in the rhizosphere is another technique that has proven useful. The successful expression of *bph* [genes involved in the degradation of polychlorinated biphenyls (PCBs)] in sugar beet using the recombinant strain *P. fluorescens* F113pcb was reported by Brazil and colleagues (1995). A reporter strain that detected 3-chlorobenzoate (3-CB), an intermediate in PCB-2 degradation, has been used to monitor the *in vivo* production of 3-CB on alfalfa roots. The authors used *gfp* fused with the *meta*-pathway Pm promoter from *P. putida* (TOL plasmid), which is strongly induced by 3-CB (Ramos *et al.*, 1986; Boldt *et al.*, 2004).

Among root exudates, numerous aromatic compounds (i.e. terpernes, flavonoids or lignin-derived components) with chemical structures similar to those of the contaminants (Fig. 2A) are released and some can act as inducers of contaminant-degradation pathways (Singer *et al.*, 2003). *L*-carvone, one of the components of spearmint root exudates, has been identified as an inducer of the genes involved in PCB degradation in *Arthrobacter* sp. strain B1B (Gilbert and Crowley, 1997). Other secondary plant metabolites, such as *p*-cymene, limonene, and the non-aromatic compound isoprene, can also induce the PCB-degradation pathway in *Arthrobacter*. Although the specific role of flavonoids as inducers of the degradation of organic pollutants has not been well established, it is known that several flavonoids sustain the growth of PCB degraders. Donnelly and colleagues (1994) reported the growth of *Ralstonia eutropha* H850 on 11 different flavonoids; *Burkholderia cepacia* LB400 on maclurin and myricetin; and *Corynebacterium* sp. MB1 on naringin, catechin, coumarin, myricetin, and *p*-coumarin among others. Most of these compounds also fostered the degradation of several PCB congeners. The degradation of flavonoids by rhizospheric bacteria leads to the formation of intermediates, including resorcinol, phloroglucinol phenylacetic acid, substituted cinnamic acids and protocatechuic acid (Pillai and Swarup, 2002; Shaw *et al.*, 2006; Fig. 2B). These compounds are likely to be mineralized through the β-ketoadipate pathway (Parke *et al.*, 2000), which is active in the catabolism of several aromatic contaminants. Protocatechuate is an intermediate in the
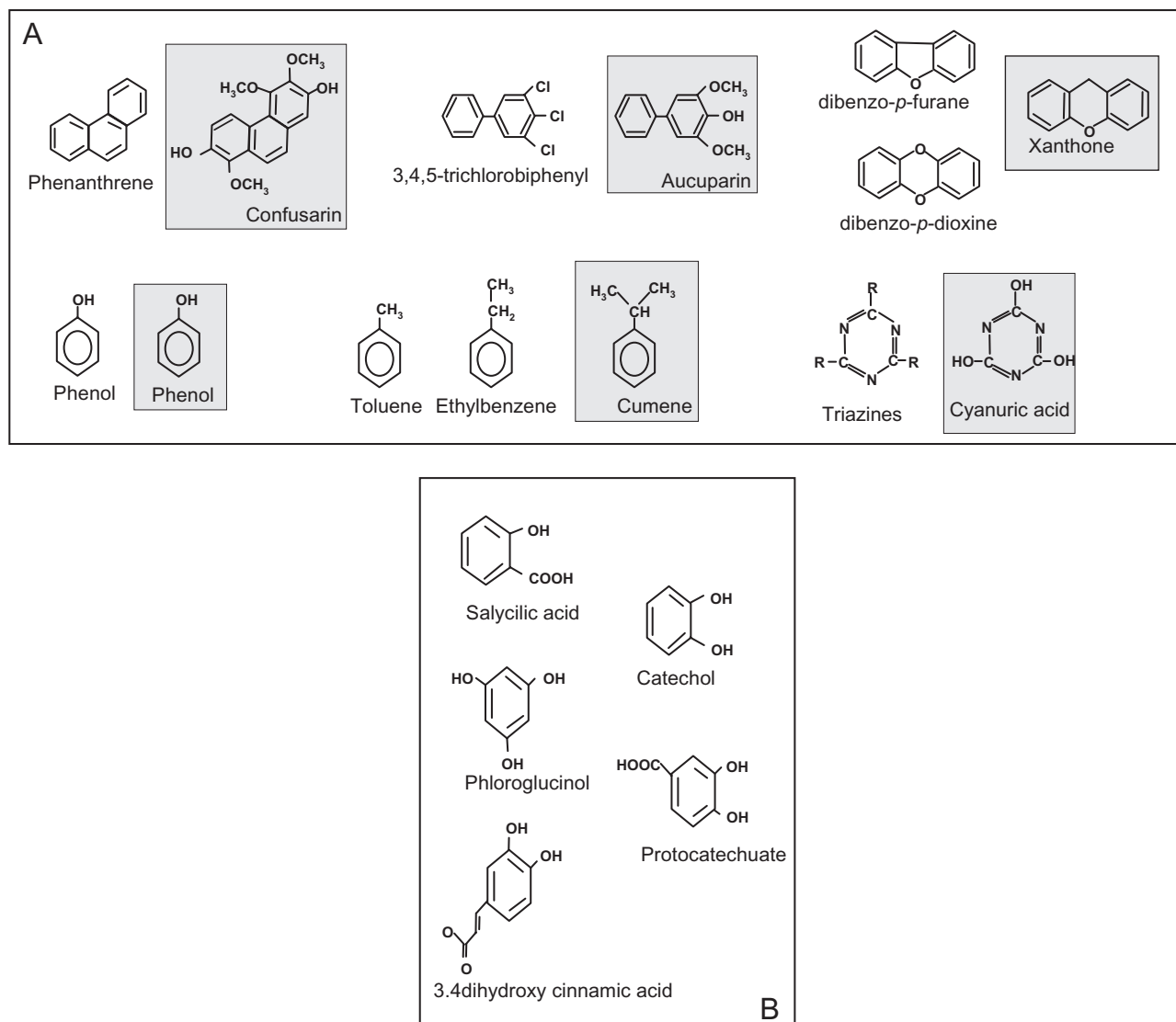
Fig. 2. A. Structural similarities among contaminants and plant products (in grey boxes).
B. The chemical structures of several intermediates in the degradation of aromatic compounds in plants and the inducers of contaminant-degradation pathways in bacteria.

degradation of polycyclic aromatic hydrocarbons (PAHs) in some microorganisms (Kim *et al.*, 2008). Salicylate, which induces systemic acquired resistance in plants, is a good inducer of the PAH-degradation pathways (Shamsuzzaman and Barnsley, 1974; Chen and Aitken, 1999). Non-aromatic plant compounds, such as linoleic acid, have also been shown to be responsible of the stimulation of pyrene and benzo[a]pyrene degradation by Gram positive bacteria (Yi and Crowley, 2007).

Although there are several reports about enhanced PAH degradation by rhizobacteria (Aprill and Sims, 1990; Miya and Firestone, 2001, Rentz *et al.*, 2004) reported that the phenanthrene-degrading activity of *P. putida* ATCC 17484 was repressed after incubation with root extracts from six different plants. Catabolite repression was the most probable cause for this repression; analysis of the root extracts indicated a minor proportion of phenolic compounds relative to other easily degradable substrates (acetate, amino acids and glucose). The apparent discrepancies between the enhanced PAH biodegradation in the rhizosphere and the inhibition of PAH degrading activity by root exudates can be explained because the rhizosphere can sustain greater numbers of degradative strains than bulk soil.

These data have led to the conclusion that for the efficient biodegradation of contaminants, the correct plant–microbe pairs must be selected, because the interactions between them are more specific than previously thought.

Improving rhizoremediation

Although the biodegradative abilities of the bacteria, and the expression and maintenance of bacterial genes in the rhizosphere are extremely important for the effective removal of contaminants in phytorhizoremediation, several other aspects can improve the effectiveness of the process.

The selection of the best plant–bacteria combination

As mentioned previously, the root exudate composition changes with the developmental stage of the plant and depends on plant species; these variations obviously exert different effects on the rhizospheric community (Smalla *et al.*, 2001; Berg *et al.*, 2002; Garbeva *et al.*, 2004). *Salix* sp. plants are used in many phytorhizoremediation experiments because they produce salicylic acid and related compounds that induce the degradation of PAHs and PCBs (de Cárcer *et al.*, 2007). Flavonoids are produced by plants as a defence mechanism against pathogens. However, plants with a higher content of flavonoids will be efficiently colonized by tolerant bacteria (Palumbo *et al.*, 1998). The root exudate composition will also favour proliferation of bacteria that will degrade them efficiently. *Pseudomonas putida* PML2 can grow using plant flavonoids (it is also a PCB degrader) and it has been demonstrated that it colonizes the rhizosphere of wild-type *Arabidopsis thaliana* (or a mutant that overproduces flavonoids) better than the rhizosphere of a mutant that does not produce flavonoids (Narasimhan *et al.*, 2003).

It is also known that the quantity and quality of the root exudates varies with stress. Nutrient or water stress induced an increase in root hair density, while phosphorous deficiency increased the release of exudates due to a reduction in the integrity of the membranes. These stresses, in turn, lead to an increase in the number of bacteria in the rhizosphere (Chaudhry *et al.*, 2005). The presence of contaminants in soils represents a stress for the plant and it could enhance the contaminant biodegradation. Root exudates can also increase the bioavailability of some contaminants. However, contaminants can also reduce biodegradation if they affect the growth of the roots. Therefore, bacteria with the capacity to promote growth (plant growth-promoting rhizobacteria) are receiving increased attention by the rhizoremediation field. This field has recently been reviewed by Arshad and colleagues (2007) and Zhuang and colleagues (2007) and so we will address the reader to these reviews for more information.

Rhizospheric communities also change the plant environment, i.e. the microbial degradation of contaminants provides a clean environment by decreasing the pollutant concentration in the area near the roots favouring plant growth and site restoration. The presence of pathogens in the soil induces the plant defence response and increases the number of phenolic compounds in the rhizosphere.

Siciliano and colleagues (2002) demonstrated that the presence of the alkane monooxygenase genes were more prevalent in endophytic and rhizospheric microbial communities than in bacteria present in bulk soil contaminated with hydrocarbons. However, the results obtained when they studied the prevalence of the xylene monooxygenase or naphthalene dioxygenase genes were the opposite; their presence was higher in bulk soil microbial communities than those near or inside the plant. This suggested that rhizospheric effects on the bacterial community also depend on the contaminant and led to the hypothesis that the effectiveness of rhizoremediation strategies correlates with the selection of the best plant–bacterium pair in each specific case (Siciliano *et al.*, 2003).

Rhizospheric bacteria can be better equipped to colonize the rhizosphere and are the best option for degradation. Shim and colleagues (2000) introduced the toluene *o*-monooxygenase genes (TOM) from *B. cepacia* G4 into several bacteria isolated from the poplar rhizosphere. The authors showed that when they introduced recombinant strains to coat poplar tree roots in non-sterile soil, recombinants that were derived from the plant rhizosphere were able to thrive, while non-rhizospheric recombinant strains were not maintained in the rhizosphere. These strains were also able to express the TOM and degrade trichloroethylene (TCE).

Endophytic bacteria

Because of the complex plant–rhizobacteria interactions, the use of endophytic bacteria for biodegradation has been extensively explored in the last years (reviewed by Doty, 2008; Ryan *et al.*, 2008). Endophytic bacteria that colonize the internal tissues of the plant without causing a negative effect (Schulz and Boyle, 2006) have less competition for nutrients and are physically protected from adverse changes in the environment (Reinhold-Hurek and Hurek, 1998). However, successful remediation by endophytic bacteria requires the transport of the contaminant to the plants' internal tissues. Contaminant transport and its distribution in plants have been reported to depend on soil and plant properties and on the physicochemical properties of the contaminants (Sung *et al.*, 2001), *a priori*, it is clear that not all pollutants will be efficiently transported to the root interior. Xenobiotics with a $\log K_{ow}$ (octanol/water partition coefficient) higher than 3.5 are likely to be absorbed by the root surface; however, plants can take up compounds with a $\log K_{ow}$ between 0.5 and 3.5 (Briggs *et al.*, 1982).

Several delivery methods for introducing endophytic bacteria into plants have been reported, including seed inoculation, soil drench, foliar spray and pruned-root dip; however, the method of choice will largely depend on the specific plant–endophyte pair to be used (Bressan and Borges, 2004; Rosenblueth and Martínez-Romero, 2006).

Pseudomonaceae, *Burkholderiaceae* and *Enterobacteriaceae* are among the most common cultivatable endophytic species isolated from a wide variety of hosts, including woody trees, herbaceous crops and grass species (Lodewyckx *et al.*, 2002). Although the biodegradative capacity of the endophytic bacteria has not been extensively investigated, reports on the ability of several endophytic bacteria to degrade some pollutants (i.e. explosives, herbicides or hydrocarbons) have been published (Van Aken *et al.*, 2004; Germaine *et al.*, 2006; Phillips *et al.*, 2008). Also, endophytic bacteria resistant to high concentrations of heavy metals, BTEX (benzene, toluene, ethyl-benzene and xylenes), TCE or PAHs have been identified (Moore *et al.*, 2006; Doty, 2008). As mentioned above, Siciliano and colleagues (2002) demonstrated that some plants can accumulate bacterial endophytic genotypes for the degradation of contaminants. In any case, the advantages of using rhizobacteria or endophytic bacteria will depend on the type of contaminant and the degradation abilities of each type of bacteria.

Seed colonization

One of the least expensive techniques that can be used to introduce microorganisms into soil is to cover the seeds with the appropriate bacteria. Similarly, the introduction of endophytes can be done following similar procedures. For this, microbes need to adhere well to the seeds (Colleran, 1997). Adhesion to seeds has been studied using classical counts of viable cells, and more recently by taking advantage of reporter genes, such as the *gfp* or *lux* genes. These assays are frequently coupled to microscopy techniques in order to facilitate the identification of target microbes. Scanning electron microscopy has also been used to track bacteria adhered to seeds. The mechanisms for the attachment of bacteria to seeds seem to be common for most biotic surfaces, including roots, and have been discussed above (i.e. flagellar and chemotaxis proteins) (Yaryura *et al.*, 2008). One of the most original approaches for studying the adhesion of bacteria to seeds was developed by Espinosa-Urgel and colleagues (2000) who designed a strategy based on the selection of mutants unable to adhere to seeds. To this end, the model microorganism *P. putida* KT2440 was mutagenized randomly with mini-Tn*5*. The pool of Km^R mutants was mixed with corn seeds placed in a syringe. Following incubation, the seeds were washed to remove bacteria that failed to adhere or which adhered loosely. By

repeating the process, the authors ended up with a number of 'enriched' mutants with limited adherence to seeds. Some of the mutants were defective in the attachment to abiotic and biotic surfaces, while others were only defective in attaching to biotic surfaces, suggesting that biofilm formation proceeds through two different mechanisms depending on whether the surface can be a source of nutrients or not (Watnick *et al.*, 1999). Motility and chemotaxis proteins were not detected during this screen, probably because under the conditions used (shaking) the bacteria do not need to move towards the seeds. In this work, as in others before, several outer membrane proteins were shown to be involved in seed adhesion (Smit *et al.*, 1992; Dörr *et al.*, 1998; Yousef-Coronado *et al.*, 2008) and this is in agreement with the fact that outer surfaces are the first contact point between a bacterium and the seed.

Production of biosurfactants

A problem for soil bioremediation is the bioavailability of the pollutant. Most organic contaminants are highly hydrophobic compounds that dissolve poorly in water and many can form complexes with soil particles. This lack of bioavailability often lowers removal efficiencies (Johnsen *et al.*, 2005). Bacteria use different strategies to promote the bioavailability of hydrophobic compounds (i.e. PAHs), including the excretion of biosurfactants, the production of extracellular polymeric substances and the formation of biofilms on PAH crystals. Biosurfactants are amphiphilic molecules that form spherical or lamellar micelles when the surfactant concentration exceeds a critical micelle concentration that is specific for each compound. Hydrophobic contaminants become solubilized in the hydrophobic cores of the micelles, which increases the transfer of the compounds from a solid to water phase where it becomes more accessible to bacteria. One important group of bacterial biosurfactants are the glycolipids of which rhamnolipids are the major representative. It has been shown that rhamnolipids are able to enhance the biodegradation rate of contaminants (Zhang and Miller, 1994; Providenti *et al.*, 1995; Shreve *et al.*, 1995; Mulligan, 2005; Cui *et al.*, 2008). Kuiper and colleagues (2004a) isolated a *P. putida* strain from plant roots at a site polluted with PAHs that produce two lipopeptide biosurfactants. These lipopeptides (named putisolvins) increased the formation of emulsions with toluene. Searching for rhizobacteria that promote the bioavailability of contaminants is therefore of great interest in the context of bioremediation. This property is also of interest because a number of biodegradative microbes exhibit positive chemotaxis towards the pollutants (Parales and Haddock, 2004). Therefore, the combined action of the biosurfactant and chemotaxis may contribute to bacterial

proliferation and microbial spread in polluted soils, leading to the clearing of more ample zones.

Engineering bacteria for rhizoremediation

The genetic modification of bacteria to improve bioremediation capacity is a classical approach. Reports about the introduction of catabolic genes into different bacteria, the construction of 'hybrid pathways', and promoter modifications to increase the expression of genes of interest, are numerous in the literature (see i.e. Ramos *et al.*, 1994). The construction of recombinant strains able to combine different traits, such as the degradation of contaminants together with the production of biosurfactants, good colonization abilities and the capacity to promote plant growth, are still desirable.

Barac and colleagues (2004) showed improved toluene phytoremediation using engineered endophytic bacteria. The authors transferred the pTOM plasmid, which encodes the toluene degradation genes, via conjugation from *B. cepacia* G4 to *B. cepacia* L.S.2.4, a natural endophyte of yellow lupine. Although the recombinant strain was not maintained in the endophytic community, there was a horizontal gene transfer of the *tom* (toluene monooxygenase) operon to different members of the endogenous endophytic community (Taghavi *et al.*, 2005), demonstrating new avenues for introducing desirable traits into the community.

Still, the release of recombinant organisms in the field is restricted in many countries and these legal limitations, together with some well sustained scientific concerns, may limit the development of this field.

Rhizoremediation studies

One of the classical papers in the field of 'natural' rhizoremediation is a report by Radwan and colleagues (1995) showing that plants growing in sand contaminated by oil spills after the Gulf War exhibited clean roots due to the removal of aromatic hydrocarbons by microorganisms. Although there are many reports about rhizoremediation experiments under laboratory conditions (reviewed by Zhuang *et al.*, 2007), there are fewer examples in the scientific literature detailing the successful removal of pollutants from contaminated soils in 'real' scenarios via the concept of 'designed' rhizoremediation.

A comprehensive number of rhizoremediation experiments have been previously listed in several reviews (Kuiper *et al.*, 2004b; Chaudhry *et al.*, 2005; Zhuang *et al.*, 2007), so we will only present a few examples that have not been included in these reports and in which different experimental approaches have been used.

Hexachlorocyclohexane is a highly persistent pollutant; its γ-isomer (lindane) has frequently been used as pesticide and is widespread throughout the biosphere. Although *Sphingomonas* UT26 is a well-characterized lindane-degrading bacterium, it was not able to proliferate in soil, probably due to its high sensitivity to the low water content in the soil. Böltner and colleagues (2008) set up a double enrichment approach (Kuiper *et al.*, 2001) in which both lindane degradation and root proliferation of bacteria were prerequisites for selection. This yielded *Sphingomonas* strains that were now able to proliferate in the plant root. Greenhouse assays revealed that up to 30% of lindane in soil can be removed in a 3-month period.

In the case of TNT removal, over 90% of TNT in the soil could be removed in field experiments through the combined action of phytoremediation and rhizoremediation. In these assays, phytoremediation proved more efficient than rhizoremediation, but bacteria played a key role in the establishment of plants at the polluted site (van Dillewijn *et al.*, 2007).

Although several PCB degraders have been identified, PCB degradation is inefficient (Donnelly *et al.*, 1994). One of the best inducers of PCB degradation is biphenyl, which obviously cannot be used as a soil amendment to promote PCB elimination. Several attempts to promote PCBs rhizoremediation via the introduction of the *bph* genes under the control of different promoters in *P. fluorescens* F113 (a strain with good colonization abilities) have had limited success (Brazil *et al.*, 1995; Villacieros *et al.*, 2005). In a different approach, Narasimhan and colleagues (2003) used the ability of *P. putida* PML2 to degrade phenylpropanoid compounds to promote PCB degradation. In *Arabidopsis thaliana,* 37% of root exudates were flavonoids and most of these were phenylpropanoids. They showed that a wild-type strain *P. putida* PML2 was able to establish in the rhizosphere of *Arabidopsis* plants better that an auxotrophic mutant that was unable to use phenylpropanoids for growth. Although both mutant and wild-type strains presented similar growth on different PCBs in liquid cultures, PCB elimination was higher when the parental strain was introduced in gnotobiotic systems with *Arabidopsis*, than when the mutant was used. Polychlorinated biphenyl elimination was inferior if an *Arabidopsis* mutant unable to produce phenylpropanoids was used. These experiments showed that rhizoengineering, the modification of microbial populations in the plant roots for biotechnological purposes, is a valuable option to enhance contaminant degradation.

Engineering proteins to be expressed at the surface of the microbial cell is another promising strategy for pollutant removal, especially for the removal of heavy metals. This strategy has recently been reviewed by Saleem and colleagues (2008) and we refer the reader to this review for more information.

Future prospects

Further studies on improving the expression of catabolic genes in the rhizosphere and in the selection of the best plant–microbe combinations will have to be translated into field strategies that can demonstrate the usefulness of this approach. The utilization of endophytes in the bio-degradation of pollutants is an emerging field that has not been widely explored. Advances in this field will have to be followed by better knowledge about the absorption and transport of the toxic chemical by plants. However, this can pose a problem if the compound is then trans-located to the shoot where it can become available to animals. The fate of contaminants should be extensively studied during phytoremediation processes to avoid undesired effects if field tests are performed. Exploring the molecular communication between plants and microbes, and exploiting this communication to achieve better results in the elimination of contaminants, is a fas-cinating area of research. These studies may reveal the mechanisms underlying microbe–plant interactions and we predict that this approach will now be adopted to study the induction of catabolic pathways in polluted soils undergoing rhizoremediation. The new '-omics' techniques will also allow the monitoring or selection of catabolic genes to improve remediation strategies (Kiely *et al.*, 2006). The improvement of metagenomic analysis will probably reveal new degradative capacities (genes) that will be worth introducing into strains with other inter-esting traits (i.e. good root colonization abilities). The signals that plant and microbes exchange when they recognize each other will have to be interpreted and the molecular basis of the specific interactions between certain plant genotypes with specific bacteria will need to be dissected. Information that can be derived from these studies may provide further insights on how to design a successful rhizoremediation strategy.

Finally, more studies about the impact of using recom-binant microorganisms over indigenous microbial commu-nities are needed to meet with safety requirements, especially with the increasing need for recombinant microbes to deal with highly toxic chemicals, such as dioxins and PCBs.

Acknowledgements

References

Aprill, W., and Sims, R.C. (1990) Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydro-carbon treatment in soil. *Chemosphere* **20:** 253–265.

Arshad, M., Saleem, M., and Hussain, S. (2007) Perspectives of bacterial ACC deaminase in phytoremediation. *Trends Biotech* **25:** 356–362.

Attila, C., Ueda, A., Cirillo, S.L.G., Chen, W., and Wood, T.K. (2008) *Pseudomonas aeruginosa* PAO1 virulence factors and poplar tree response in the rhizosphere. *Microbial Biotechnol* **1:** 17–29.

Barac, T., Taghavi, S., Borremans, B., Provoost, A., Oeyen, L., Colpaert, J.V., *et al.* (2004) Engineered endophytic bac-teria improve phytoremediation of water-soluble, volatile, organic pollutants. *Nature Biotech* **22:** 583–588.

Barr, M., East, A.K., Leonard, M., Mauchline, T.H., and Poole, P.S. (2008) *In vivo* expression technology (IVET) selection of genes of *Rhizobium leguminosarum* biovar *viciae* A34 expressed in the rhizosphere. *FEMS Microbiol Lett* **282:** 219–227.

Bender, C., Rangaswamy, V., and Loper, J. (1999) Polyketide production by plant-associated pseudomonads. *Annu Rev Phytopathol* **37:** 175–196.

Bending, G.D., Shaw, E., and Walker, A. (2001) Spatial het-erogeneity in the metabolism and dynamics of isoproturon degrading microbial communities in soil. *Biol Fert Soils* **33:** 484–489.

Berg, G., Roskot, N., Steidle, A., Eberl, L., Zock, A., and Smalla, K. (2002) Plant-dependent genotypic and pheno-typic diversity of antagonistic rhizobacteria isolated from different *Verticillum* host plants. *Appl Environ Microbiol* **68:** 3328–3338.

Boldt, T.S., Sørensen, J., Karlson, U., Molin, S., and Ramos, C. (2004) Combined use of different Gfp reporters for moni-toring single-cell activities of a genetically modified PCB degrader in the rhizosphere of alfalfa. *FEMS Microbiol Ecol* **48:** 139–148.

Brazil, G.M., Kenefick, L., Callanan, M., Haro, A., de Lorenzo, V., Dowling, D.N., and O'Gara, F. (1995) Construction of a rhizosphere Pseudomonad with potential to degrade poly-chlorinated biphenyls and detection of *bph* gene expression in the rhizosphere. *Appl Environ Microbiol* **61:** 1846–1952.

Bressan, W., and Borges, M.T. (2004) Delivery methods for introducing endophytic bacteria into maize. *BioControl* **49:** 315–322.

Briggs, G.G., Bromilow, R.H., and Evans, A.A. (1982) Rela-tionship between lipophilicity and root uptake and translo-cation of non-ionized chemicals by barley. *Pestic Sci* **13:** 495–504.

Broek, A.V., Lambrecht, M., and Vanderleyden, J. (1998) Bacterial chemotactic motility is important for the initiation of wheat root colonization by *Azospirillum brasilense*. *Microbiology* **144:** 2599–2606.

Burken, J.G. (2004) Uptake and metabolism of organic com-pounds: green-liver model. In *On Phytoremediation*. McCutcheon, J.L., and Schnoor, S.D. (eds). Hoboken, NJ, USA: John Wiley & Sons, pp. 59–84.

Böltner, D., Godoy, P., Muñoz-Rojas, J., Duque, E., Moreno-Morillas, S., Sánchez, L., and Ramos, J.L. (2008) Rhizore-mediation of lindane by root-colonizing *Sphingomonas*. *Microbial Biotech* **1:** 87–93.

Campbell, R., and Greaves, M.P. (1990) Anatomy and com-munity structure of the rhizosphere. In *The Rhizosphere*. Lynched, J.M. (ed). Chichester, UK: John Wiley & Sons, pp. 11–34.

Capdevila, S., Martínez-Granero, F.M., Sánchez-Contreras, M., Rivilla, R., and Martín, M. (2004) Analysis of *Pseudomonas fluorescens* F113 genes implicated in flagellar filament synthesis and their role in competitive root colonization. *Microbiology* **150:** 3889–3897.

de Cárcer, D., Martín, M., Karlson, U., and Rivilla, R. (2007) Changes in bacterial populations and in biphenyl dioxygenase gene diversity in a polychlorinated biphenyl-polluted soil after introduction of willow trees for rhizoremediation. *Appl Environ Microbiol* **73:** 6224–6232.

Cerniglia, C.E. (1993) Biodegradation of polycyclic aromatic hydrocarbons. *Curr Opin Biotechnol* **4:** 331–338.

Chaudhry, Q., Blom-Zandstra, M., Gupta, S., and Joner, E.J. (2005) Utilising the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. *Environ Sci Pollut Res* **12:** 34–48.

Chen, M.M., Zhu, Y.G., Su, Y.H., Chen, B.D., Fu, B.J., and Marschner, P. (2006) Effects of soil mixture and plant interactions on the soil microbial community structure. *Eur J Soil Biol* **43:** 31–38.

Chen, S.-H., and Aitken, M.D. (1999) Salicylate stimulates the degradation of high-molecular weight polycyclic aromatic hydrocarbons by *Pseudomonas saccharophila* P15. *Environ Sci Technol* **33:** 435–439.

Colleran, E. (1997) Uses of bacteria in biorremediation. In *Methods in Biotechnology, Vol. 2. Bioremediation Protocols*. Sheehan, D. (ed.). Totowa, NJ, USA: Humana Press.

Cui, C.Z., Zeng, C., Wan, X., Chen, D., Zhang, J.Y., and Shen, P. (2008) Effect of rhamnolipids on degradation of anthracene by two newly isolated strains, *Sphingomonas* sp. 12A and *Pseudomonas* sp. 12B. *J Microbiol Biotechnol* **18:** 63–66.

Cunningham, S.D., and Ow, D.W. (1996) Promises and prospects of phytoremediation. *Plant Physiol* **110:** 715–719.

Cunningham, S.D., Anderson, T.A., Schwab, A.P., and Hsu, F.C. (1996) Phytoremediation of soils contaminated with organic pollutants. *Adv Agron* **56:** 55–114.

Daane, L.L., Harjono, I., Zylstra, G.J., and Häggblom, M.M. (2001) Isolation and characterization of polycyclic aromatic hydrocarbon-degrading bacteria associated with the rhizosphere of salt marsh plants. *Appl Environ Microbiol* **67:** 2683–2691.

Danhorn, T., and Fuqua, C. (2007) Biofilm formation by plant-associated bacteria. *Annu Rev Microbiol* **61:** 401–422.

Dekkers, L.C., van der Bij, A.J., Mulders, I.H.M., Phoelich, C.C., Wentwoord, R.A.R., Glandorf, D.C.M., *et al.* (1998a) Role of the O-antigen of lipopolysaccharide, and possible roles of growth rate and NADH:ubiquinone oxidoreductase (nuo) in competitive tomato root-tip colonization by *Pseudomonas fluorescens* WCS365. *Mol Plant Microbe Interact* **11:** 763–771.

Dekkers, L.C., Bloemendaal, C.J.P., de Weger, L.A., Wijffelman, C.A., Spaink, H.P., and Lugtenberg, B.J.J. (1998b) A two-component system plays an important role in the root-colonizing ability of *Pseudomonas fluorescens* strain WCS365. *Mol Plant Microbe Interact* **11:** 45–56.

van Dillewijn, P. (2008) What gets turned on in the rhizosphere?. *Microbial Biotech* **1:** 341–342.

van Dillewijn, P., Caballero, A., Paz, J.A., González-Pérez, M.M., Oliva, J.M., and Ramos, J.L. (2007) Bioremediation of 2,4,6-trinitrotoluene under field conditions. *Environ Sci Technol* **41:** 1378–1383.

van Dillewijn, P., Couselo, J.L., Corredoira, E., Delgado, A., Wittich, R.M., Ballester, A., and Ramos, J.L. (2008) Bioremediation of 2,4,6-trinitrotoluene by bacterial nitroreductase expressing transgenic aspen. *Environ Sci Technol* **42:** 7405–7410.

Donnelly, P.K., Hegde, R.S., and Fletcher, J.S. (1994) Growth of PCB-degrading bacteria on compounds from photosynthetic plants. *Chemosphere* **28:** 981–988.

Doty, S.L. (2008) Enhancing phytoremediation through the use of transgenics and endophytes. *New Phytol* **179:** 318–333.

Dörr, J., Hurek, T., and Reinhold-Hurek, B. (1998) Type IV pili are involved in plant-microbe and fungus-microbe interactions. *Mol Microbiol* **30:** 7–17.

Espinosa-Urgel, M., Salido, A., and Ramos, J.L. (2000) Genetic analysis of functions involved in adhesion of *Pseudomonas putida* to seeds. *J Bacteriol* **182:** 2363–2369.

Fletcher, J.S., and Hedge, R.S. (1995) Release of phenols by perennial plant roots and their potential importance in bioremediation. *Environ Toxicol Chem* **31:** 3009–3016.

Garbeva, P., van Veen, J.A., and van Elsas, J.D. (2004) Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Ann Rev Phytopathol* **42:** 243–270.

Germaine, K.J., Liu, X., Cabellos, G.G., Hogan, J.P., Ryan, D., and Dowling, D.N. (2006) Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. *FEMS Microbiol Ecol* **57:** 302–310.

Gilbert, E.S., and Crowley, D.E. (1997) Plant compounds that induce polychlorinated biphenyl biodegradation by A*rthrobacter* sp. strain B1B. *Appl Environ Microbiol* **63:** 1933–1038.

Goldstein, R.M., Mallory, L.M., and Alexander, M. (1985) Reasons for possible failure of inoculation to enhance biodegradation. *Appl Environ Microbiol* **50:** 977–983.

Haas, D., and Défago, G. (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat Rev Microbiol* **3:** 307–319.

Handelsman, J., and Stabb, E.V. (1996) Biocontrol of soil-borne plant pathogens. *Plant Cell* **8:** 1855–1869.

Hartmann, A., Schmid, M., van Tuinen, D., and Berg, G. (2009) Plant-driven selection of microbes. *Plant Soil* online DOI 10.1007/s11104-008-9814-y.

Head, M. (1998) Bioremediation: towards a credible technology. *Microbiology* **144:** 599–608.

Hiltner, L. (1904) Uber neue erfahrungen und probleme auf dem gebiet der bodenbakteriolgie und unter besonderes berucksichtigung der grundugungen und brauche. *Arb Dtsch Landwirt Ges Berl* **98:** 59–78.

Hughes, J.B., Shanks, J., Vanderford, M., Lauritzen, J., and Bhadra, R. (1997) Transformation of TNT by aquatic plants and plant tissue cultures. *Environ Sci Technol* **31:** 266–271.

Johnsen, A.R., Wick, L.Y., and Harms, H. (2005) Principles of microbial PAH-degradation in soil. *Environ Pollut* **133:** 71–84.

Jussila, M.M., Jurgens, G., Lindström, K., and Suominen, L. (2006) Genetic diversity of culturable bacteria in oil-contaminated rhizosphere of *Galega orientalis*. *Environ Pollut* **139:** 244–257.

Kent, A.D., and Triplett, E.W. (2002) Microbial communities and their interactions in soil and rhizosphere ecosystems. *Annu Rev Microbiol* **56:** 211–236.

Kielak, A., Pijl, A.S., van Veen, J.A., and Kowalchuk, G.A. (2008) Differences in vegetation composition and plant species identity lead to only minor changes in soil-borne microbial communities in a former arable field. *FEMS Microbiol Ecol* **63:** 372–382.

Kiely, P.D., Haynes, J.M., Higgins, C.H., Franks, A., Mark, G.L., Morrissey, J.P., and O'Gara, F. (2006) Exploiting new systems-based strategies to elucidate plant-bacterial interactions in the rhizosphere. *Microb Ecol* **51:** 257–266.

Kim, S.J., Kweon, O., Jones, R.C., Edmondson, R.D., and Cerniglia, C.E. (2008) Genomic analysis of polycyclic aromatic hydrocarbon degradation in *Mycobacterium vanbaalenii* PYR-1. *Biodegradation* **19:** 859–881.

Kuiper, I., Bloemberg, G.V., and Lugtenberg, B.J.J. (2001) Selection of a plant-bacterium pair as a novel tool for rhizostimulation of polycyclic aromatic hydrocarbon-degrading bacteria. *Mol Plant Microbe Interact* **14:** 1197–1205.

Kuiper, I., Lagendijk, E.L., Bloemberg, G.V., and Lugtenberg, B.J.J. (2004b) Rhizoremediation: a beneficial plant-microbe interaction. *Mol Plant Microbe Interact* **17:** 6–15.

Kuiper, I., Lagendijk, E.L., Pickford, R., Derrick, J.P., Lamers, G.E.M., Thomas-Oates, J.E., *et al.* (2004a) Characterization of two *Pseudomonas putida* lipopeptide biosurfactants, putisolvin I and II, which inhibit biofilm formation and break down existing biofilms. *Mol Microbiol* **51:** 97–113.

Levanon, D. (1993) Roles of fungi and bacteria in the mineralization of the pesticides atrazine, alachlor, malathion and carbofuran in soil. *Soil Biol Biochem* **25:** 1097–1105.

Lodewyckx, C., Vangronsveld, J., Porteous, F., Moore, E.R.B., Taghavi, S., Mergeay, M., and van der Lelie, D. (2002) Endophytic bacteria and their potential applications. *Crit Rev Plant Sci* **21:** 583–606.

Lugtenberg, B.J.J., and Dekkers, L.C. (1999) What makes *Pseudomonas* bacteria rhizosphere competent?. *Environ Microbiol* **1:** 9–13.

Lugtenberg, B.J.J., de Weger, L.A., and Bennett, J.W. (1991) Microbial stimulation of plant growth protection from disease. *Curr Biotechnol* **2:** 457–465.

Lugtenberg, B.J.J., Chin-A-Woeng, T.F.C., and Bloemberg, G.V. (2002) Microbe-plant interactions: principles and mechanisms. *Antonie van Leeuwenhoek* **81:** 373–383.

Lübeck, P.S., Hansen, M., and Sørensen, J. (2000) Simultaneous detection of the establishment of seed-inoculated *Pseudomonas fluorescens* strain DR54 and native soil bacteria on sugar beet root surfaces using fluorescence antibody and *in situ* hybridization techniques. *FEMS Microbiol Ecol* **33:** 11–19.

Madsen, E.L. (2006) The use of stable isotope probing techniques in bioreactor and field studies on bioremediation. *Curr Opin Biotechnol* **17:** 92–97.

Mark, G.L., Dow, J.M., Kiely, P.D., Higgins, H., Haynes, J., Baysse, C., *et al.* (2005) Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe-plant interactions. *Proc Natl Acad Sci USA* **102:** 17454–17459.

Marschner, P., Yang, C-H., Lieberei, R., and Crowley, D.E. (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. *Soil Biol Biochem* **33:** 1437–1445.

Martínez-Granero, F., Rivilla, R., and Martín, M. (2006) Rhizosphere selection of highly motile phenotypic variants of *Pseudomonas fluorescens* with enhanced competitive colonization ability. *Appl Environ Microbiol* **72:** 3429–3434.

Matilla, M.A., Espinosa-Urgel, M., Rodríguez-Herva, J.J., Ramos, J.L., and Ramos-González, M.I. (2007) Genomic analysis reveals the major driving forces of bacterial life in the rhizosphere. *Genome Biol* **8:** R179.

Merckx, R., van Ginkel, J.H., Sinnaeve, J., and Cremers, A. (1986) Plant-induced changes in the rhizosphere of maize and wheat. I. Production and turnover of root-derived material in the rhizosphere of maize and wheat. *Plant Soil* **96:** 85–93.

Miya, R.K., and Firestone, M.K. (2001) Enhanced phenanthrene biodegradation in soil by slender oat root exudates and root debris. *J Environ Qual* **30:** 1911–1918.

Moore, F.P., Barac, T., Borremans, B., Oeyen, L., Vangronsveld, J., Campbell, van der Lelie, D., *et al.* (2006) Endophytic bacterial diversity in poplar trees growing on a BTEX-contaminated site: the characterisation of isolates with potential to enhance phytoremediation. *Syst Appl Microbiol* **29:** 539–556.

Morgan, J.A.W., Bending, G.D., and White, P.J. (2005) Biological costs and benefits to plant–microbe interactions in the rhizosphere. *J Exp Bot* **56:** 1729–1739.

Mulligan, C.N. (2005) Environmental applications for biosurfactants. *Environ Pollut* **133:** 183–198.

Narasimhan, K., Basheer, C., Bajic, V.B., and Swarup, S. (2003) Enhancement of plant-microbe interactions using a rhizosphere metabolomics-driven approach and its application in the removal of polychlorinated biphenyls. *Plant Physiol* **132:** 146–153.

Palumbo, J.D., Kado, C.I., and Phillips, D.A. (1998) An isoflavonoid-inducible efflux pump in *Agrobacterium tumefaciens* is involved in competitive colonization of roots. *J Bacteriol* **180:** 3107–3113.

Parales, R.E., and Haddock, J.D. (2004) Biocatalytic degradation of pollutants. *Curr Opin Biotecnol* **15:** 374–379.

Parke, D., D'Argenio, D.A., and Ornston, L.N. (2000) Bacteria are not what they eat: that is why they are so diverse. *J Bacteriol* **182:** 257–263.

Parkin, T.B., and Shelton, D.R. (1992) Spatial and temporal variability of carbofuran degradation in soil. *J Environ Qual* **21:** 672–678.

Phillips, L.A., Germida, J.J., Farrell, R.E., and Greer, C.W. (2008) Hydrocarbon degradation potential and activity of endophytic bacteria associated with prairie plants. *Soil Biol Biochem* **40:** 3054–3064.

Pillai, B.V.S., and Swarup, S. (2002) Elucidation of the flavonoid catabolism pathway in *Pseudomonas putida* PML2 by comparative metabolic profiling. *Appl Environ Microbiol* **68:** 143–151.

Providenti, M.A., Flemming, C.A., Lee, H., and Trevors, J.T. (1995) Effect of addition of rhamnolipid biosurfactant

or rhamnolipid-producing *Pseudomonas aeruginosa* on phenanthrene mineralization in soil slurries. *FEMS Microbiol Ecol* **17:** 15–26.

Radwan, S., Sorkhoh, N., and el-Nemr, I. (1995) Oil biodegradation around roots. *Nature* **376:** 302.

Rainey, P.B. (1999) Adaptation of *Pseudomonas fluorescens* to the plant rhizosphere. *Environ Microbiol* **1:** 243–257.

Rainey, P.B., and Preston, G.M. (2000) In vivo expression technology strategies: valuable tools for biotechnology. *Curr Opin Biotechnol* **11:** 440–444.

Ramos, C., Mølbak, L., and Molin, S. (2000a) Bacterial activity in the rhizosphere analyzed at the single-cell level by monitoring ribosome contents and synthesis rates. *Appl Environ Microbiol* **66:** 801–809.

Ramos, C., Molina, L., Mølbak, L., Ramos, J.L., and Molin, S. (2000b) A bioluminescent derivative of *Pseudomonas putida* KT2440 for deliberate release into the environment. *FEMS Microbiol Ecol* **34:** 91–102.

Ramos, C., Licht, T.R., Sternberg, C., Krogfelt, K.A., and Molin, S. (2001) Monitoring bacterial growth activity in biofilms from laboratory flow Chambers, plant rhizosphere, and animal intestine. *Methods Enzymol* **337:** 21–42.

Ramos, J.L., Díaz, E., Dowling, D., de Lorenzo, V., Molin, S., O'Gara, F., *et al.* (1994) The behaviour of bacteria designed for biodegradation. *Biotechnology (NY)* **13:** 1349–1356.

Ramos, J.L., Stolz, A., Reineke, W., and Timmis, K.N. (1986) Altered effector specificities in regulators of gene expression: TOL plasmid *xylS* mutants and their use to engineer expansion of the range of aromatics degraded by bacteria. *Proc Natl Acad Sci USA* **83:** 8467–8471.

Ramos-González, M.I., Campos, M.J., and Ramos, J.L. (2005) Analysis of *Pseudomonas putida* KT2440 gene expression in the maize rhizosphere: in vivo expression technology capture and identification of root-activated promoters. *J Bacteriol* **187:** 4033–4041.

Rasmussen, J., Jensen, P.H., Holm, P.E., and Jacobsen, O.S. (2004) Method for rapid screening of pesticide mineralization in soil. *J Microbiol Methods* **57:** 151–156.

Rattray, E.A.S., Prosser, J.I., Glover, L.A., and Killham, K. (1995) Characterization of rhizosphere colonization by luminescent *Enterobacter cloacae* at the population and single-cells levels. *Appl Environ Microbiol* **61:** 2950–2957.

Rediers, H., Rainey, P.B., Vanderleyden, J., and De Mot, R. (2005) Unraveling the secret lives of bacteria: use of in vivo expression technology and differential fluorescence induction promoter traps as tools for exploring niche-specific gene expression. *Microbiol Mol Biol Rev* **69:** 217–2661.

Reinhold-Hurek, B., and Hurek, T. (1998) Interactions of gramineous plants with Azoarcus spp. and other diazotrophs: identification, localization and perspectives to study their function. *Crit Rev Plant Sci* **17:** 29–54.

Rentz, J.A., Alvarez, P.J.J., and Schnoor, J.L. (2004) Repression of *Pseudomonas putida* phenanthrene-degrading activity by plant root extracts and exudates. *Environ Microbiol* **6:** 574–583.

Rodríguez-Navarro, D.N., Dardanelli, M.S., and Ruíz-Saínz, J.E. (2007) Attachment of bacteria to the roots of higher plants. *FEMS Microbiol Lett* **272:** 127–136.

Rosenblueth, M., and Martínez-Romero, E. (2006) Bacterial endophytes and their interactions with hosts. *Mol Plant Microbe Interact* **19:** 827–837.

Rovira, A.D. (1965) Interactions between plant roots and soil microorganisms. *Annu Rev Microbiol* **19:** 241–246.

Ryan, R.P., Germaine, K., Franks, A., Ryan, D.J., and Dowling, D.N. (2008) Bacterial endophytes: recent developments and applications. *FEMS Microbiol Lett* **278:** 1–9.

Saleem, M., Brim, H., Hussaing, S., Arsahed, M., Leigh, M.B., and Zia-ul-hassan (2008) Perspectives on microbial cell surface display in bioremediation. *Biotech Adv* **26:** 151–161.

Salt, D.E., Smith, R.D., and Raskin, I. (1998) Phytoremediation. *Annu Rev Plant Physiol Plant Mol Biol* **49:** 643–668.

Sánchez-Contreras, M., Martín, M., Villacieros, M., O'Gara, F., Bonilla, I., and Rivilla, R. (2002) Phenotypic selection and phase variation occur during alfalfa root colonization by *Pseudomonas fluorescens* F113. *J Bacteriol* **184:** 1587–1596.

Schulz, B., and Boyle, C. (2006) What are endophytes? In *Microbial Root Endophytes*. Schulz, B.J.E., Boyle, C.J.C., and Sieber, T.N. (eds). Berlin, Germany: Springer-Verlag, pp. 1–13.

Shamsuzzaman, K.M., and Barnsley, E.A. (1974) The regulation of naphtalene oxygenase in pseudomonads. *J Gen Microbiol* **83:** 165–170.

Shaw, L.J., Morris, P., and Hooker, J.E. (2006) Perception and modification of plant flavonoid signals by rhizosphere microorganisms. *Environ Microbiol* **8:** 1867–1880.

Shim, H., Chauhan, S., Ryoo, D., Bowers, K., Thomas, S.M., Canada, K.A., *et al.* (2000) Rhizosphere competitiveness of trichloroethylene-degrading, poplar-colonizing recombinant bacteria. *Appl Environ Microbiol* **66:** 4673–4678.

Shreve, G.S., Inguva, S., and Gunnam, S. (1995) Rhamnolipid biosurfactant enhancement of hexadecane biodegradation by *Pseudomonas aeruginosa*. *Mol Mar Biol Biotech* **4:** 331–337.

Shurtliff, M.M., Parkin, G.F., Weathers, L.J., and Gibson, D.T. (1996) Biotransformation of trichloroethylene by a phenol-induced mixed culture. *J Environ Eng* **122:** 581–589.

Siciliano, S.D., Fortin, N., Mihoc, A., Wisse, G., Labelle, S., Beaumier, D., *et al.* (2002) Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. *Appl Environ Microbiol* **67:** 2469–2475.

Siciliano, S.D., Germida, J.J., Banks, K., and Greer, C.W. (2003) Changes in microbial community composition and function during a polyaromatic hydrocarbon phytoremediation field trial. *Appl Environ Microbiol* **69:** 483–489.

Singer, A.C., Crowley, D.E., and Thompson, I.P. (2003) Secondary plant metabolites in phytoremediation and biotransformation. *Trends Biotech* **21:** 123–130.

Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., *et al.* (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. *Appl Environ Microbiol* **67:** 4742–4651.

Smit, E., Leeflang, P., Gommans, S., van den Broek, J., van Mil, S., and Wernars, K. (2001) Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation

and molecular methods. *Appl Environ Microbiol* **67:** 2284–2291.

Smit, G., Swart, S., Lugtenberg, B.J.J., and Kijne, J.W. (1992) Molecular mechanisms of attachment of *Rhizobium* bacteria to plant roots. *Mol Microbiol* **6:** 2897–2903.

Sticher, L., Mauch-Mani, B., and Métraux, J.P. (1997) Systemic acquired resistance. *Annu Rev Phytopathol* **35:** 235–270.

Sung, K., Corapcioglu, M.Y., Drew, M.C., and Munster, C.L. (2001) Plant and Environmental Interactions: plant contamination by organic pollutants in phytoremediation. *J Environ Qual* **30:** 2081–2090.

Susarla, S., Medina, V.F., and McCutcheon, S.C. (2002) Phytoremediation: an ecological solution to organic chemical contamination. *Ecol Eng* **18:** 647–658.

Taghavi, S., Barac, T., Greenberg, B., Borremans, B., Vangronsveld, J., and van der Lelie, D. (2005) Horizontal gene transfer to endogenous endophytic bacteria from poplar improves phytoremediation of toluene. *Appl Environ Microbiol* **71:** 8500–8505.

Tian, B., Yang, J., and Zhang, K.-Q. (2007) Bacteria used in the biological control of plant-parasitic nematodes: populations, mechanisms of action, and future prospects. *FEMS Microbiol Ecol* **61:** 197–213.

Tombolini, R., van der Gaag, D.J., Gerhardson, B., and Jansson, J.K. (1999) Colonization pattern of the biocontrol strain *Pseudomonas chlororaphis* MA 342 on barley seeds visualized by using green fluorescent protein. *Appl Environ Microbiol* **65:** 3674–3680.

Trenck, v.d. TH., and Sandermann, H., Jr. (1980) Oxygenation of benzo[a]pyrene by plant microsomal fractions. *FEBS Lett* **119:** 227–231.

Urbance, J.W., Cole, J., Saxman, P., and Tiedje, J.M. (2003) BSD: the biodegradative strain database. *Nucleic Acids Res* **31:** 152–155.

Van Aken, B. (2008) Transgenic plants for phytoremediation: helping nature to clean up environmental pollution. *Trends Biotechnol* **26:** 225–227.

Van Aken, B., Yoon, J.M., and Schnoor, J.L. (2004) Biodegradation of nitro-substituted explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5-tetrazocine by a phytosymbiotic Methylobacterium sp. associated with poplar tissues (Populus deltoides x nigra DN34). *Appl Environ Microbiol* **70:** 508–517.

van Veen, J.A., van Overbeek, L.S., and van Elsas, J.D. (1997) Fate and activity of microorganisms introduced into soil. *Microbiol Mol Biol Rev* **61:** 121–135.

Villacieros, M., Whelan, C., Mackova, M., Molgaard, J., Sanchez-Contreras, M., Lloret, J., *et al.* (2005) Polychlorinated biphenyl rhizoremediation by *Pseudomonas fluorescens* F113 derivatives, using a *Sinorhizobium meliloti* nod system to drive *bph* gene expression. *Appl Environ Microbiol* **71:** 2687–2694.

Walker, T.S., Pal Bais, H.P., Grotewold, E., and Vivanco, J.M. (2003) Root exudation and rhizosphere biology. *Plant Physiol* **132:** 44–51.

Watnick, P.I., Fullner, K.J., and Kolter, R. (1999) A role for the mannose-sensitive hemagglutinin in biofilm formation by *Vibrio cholerae* El Tor. *J Bacteriol* **181:** 3606–3609.

de Weert, S., Vermeiren, H., Mulders, I.H.M., Kuiper, I., Hendrickx, N., Bloemberg, G.V., *et al.* (2002) Flagella-driven chemotaxis towards exudate components is an important trait for tomato root colonization by *Pseudomonas fluorescens*. *Mol Plant Microbe Interact* **15:** 1173–1180.

de Weger, L.A., van der Vlugt, C.I.M., Wijfjes, A.H.M., Bakker, P.A.H.M., Schippers, B., and Lugtenberg, B. (1987) Flagella of plant growth-stimulating *Pseudomonas fluorescens* strain are required for colonization of potato roots. *J Bacteriol* **169:** 2769–2773.

Yaryura, P.M., León, M., Correa, O.S., Kerber, N.L., Pucheu, N.L., and García, A.F. (2008) Assessment of the role of chemotaxis and biofilm formation as requirements for colonization of roots and seeds of soybean plants by *Bacillus amyloliquefaciens* BNM339. *Curr Microbiol* **56:** 625–632.

Yi, H., and Crowley, D.E. (2007) Bioestimulation of PAH degradation with plants containing high concentrations of linoleic acid. *Environ Sci Technol* **41:** 4382–4388.

Yousef-Coronado, F., Travieso, M.L., and Espinosa-Urgel, M. (2008) Different, overlapping mechanisms for colonization of abiotic and plant surfaces by *Pseudomonas putida*. *FEMS Microbiol Lett* **288:** 118–124.

Zhang, Y., and Miller, R.M. (1994) Effect of a Pseudomonas rhamnolipid biosurfactant on cell hydrophobicity and biodegradation of octadecane. *Appl Environ Microbiol* **60:** 2101–2106.

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