



Removal of Hydrocarbons and Other Related Chemicals Via the Rhizosphere of Plants

Lázaro Molina, Pieter van Dillewijn, Estrella Duque, Craig Daniels, Tino Krell, Manuel Espinosa-Urgel, María Isabel Ramos-González, Sara Rodríguez-Conde, Miguel A. Matilla, Regina Wittich, Juan Luis Ramos, and Ana Segura

Contents

1	Introduction	2
2	Phytoremediation Strategies	3
3	Rhizoremediation	4
3.1	Rhizoremediation Requirements	4
3.2	Rhizoremediation Experiments	7
3.3	Monitoring Rhizoremediation	8
4	Improving Rhizoremediation: Research Needs	9
	References	10

L. Molina · P. van Dillewijn · E. Duque · T. Krell · M. Espinosa-Urgel · M. I. Ramos-González · S. Rodríguez-Conde · M. A. Matilla · R. Wittich · J. L. Ramos · A. Segura (✉)
Department of Environmental Protection, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Granada, Spain
e-mail: lazaro.molina@cnb.csic.es; pieter.vandillewijn@eez.csic.es; estrella.duque@eez.csic.es; tino.krell@eez.csic.es; t.krell@eez.csic.es; manuel.espinosa@eez.csic.es; maribel.ramos@eez.csic.es; sara.rodriguez.conde@gmail.com; miguel.matilla@eez.csic.es; regina.wittich@eez.csic.es; jlramos@eez.csic.es; juanluis.ramos@eez.csic.es; ana.segura@eez.csic.es

C. Daniels
Department of Environmental Protection, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Granada, Spain

Developmental and Stem Cell Biology Program, Brain Tumour Research Centre,
The Hospital for Sick Children, Toronto, ON, Canada
e-mail: craig.daniels@sickkids.ca

© Springer International Publishing AG, part of Springer Nature 2018

R. Steffan (ed.), *Consequences of Microbial Interactions with Hydrocarbons, Oils, and Lipids: Biodegradation and Bioremediation*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-319-44535-9_10-1

Abstract

Recalcitrant organic toxic chemicals have been accumulating for decades as a consequence of industrial activity. Concerns about environmental contamination have been rising in the last three decades and therefore the need for soil remediation has become a priority. Although physicochemical techniques are currently the most efficient methods being used to remove contaminants, they are very expensive and therefore impractical in many locations. The use of plants in the bioremediation of soils has been proposed as an attractive strategy; however, plants lack the extraordinary biodegradative capabilities of microorganisms. Consequently rhizoremediation, a technology which combines microorganisms that eliminate contaminants in the plant roots which provide nutrients for these microorganisms, has emerged. To design a successful rhizoremediation strategy, microorganisms need to proliferate in the root system, and the bacterial catabolic pathways have to be operative. Recent advances in these aspects, together with some techniques to improve biodegradation in the rhizosphere will be presented in this chapter.

1 Introduction

Industrialization has allowed human development and higher living standards; however, these benefits have come at a high price in environmental terms. The inappropriate use of fertilizers and pesticides to improve crop production to cope with the needs of a growing population is leading to the contamination of underground water by nitrates and to the pollution of soil and surface water. The greater energy needs of society have increased CO₂ production which is now one of the main concerns for governments. Industrial waste has resulted in the accumulation of recalcitrant organic chemicals such as DDT, lindane, triazines, and polychlorinated biphenyls (PCBs). In addition, soils are being gradually affected by the deposition of fuel particles enriched in polycyclic aromatic hydrocarbons (PAHs), as well as benzene and other chemicals derived from petrochemical activities. Recently, an increase in contaminants derived from personal care products and pharmaceuticals (PCPPs) has been detected in water. All these contaminants provoke an adverse effect on ecosystems and are a serious threat to human health. Due to their toxicity and in many cases their carcinogenic, teratogenic, endocrine disruption and mutagenic properties, a number of these chemicals have been declared pollutants and their removal is a priority for Environmental Protection Agencies. The removal of pollutants from air, water, and soil requires different technologies, and in this chapter, we will focus on the removal of contaminants from soil. Removal of pollutants from soil is a very complex and expensive procedure as it depends on numerous uncontrollable factors. Most of the successful remediation examples have included the physical removal of the contaminated soil (excavation, truck, and dump) and/or physical and/or chemical treatments; however, these techniques are quite expensive and involve the disruption of the soil structure (Gómez-Sagasti et al. 2016). In general, biological treatments are cheaper than physicochemical ones; however, they have

been used in recent years with different degrees of success (Segura and Ramos 2013). The success of biological pollutant removal depends on the particular bioremediation strategy, the soil properties, age of the contamination, initial concentration of the contaminants, and obviously on the contaminant's toxicity (dos Santos and Maranhão 2018). *On-site* techniques such as land-farming or composting are promising options, but they involve the manipulation of soils and sometimes provoke the mobilization of the contaminant. *In situ* techniques, such as inoculation with microorganisms encoding the desired catalytic properties or bioaugmentation with native populations, are unreliable (Colleran 1997) and improving them by soil fertilization is expensive.

2 Phytoremediation Strategies

As an alternative to the failure of bioaugmentation, phytoremediation, the utilization of plants for the removal of pollutants, has been proposed as an attractive strategy to achieve the efficient elimination of pollutants.

The generic term, phytoremediation, is used to describe the elimination of contaminants by the plants themselves or in combination with root colonizing microorganisms, and it encompasses different strategies (Salt et al. 1998). In phytostabilization, plants either physically or by the action of the root exudates, help to sequester the contaminant to the soil particles thus making them less bioavailable (Burges et al. 2017); phytovolatilization is the term used when plants take up the contaminant from the soil and transform it into a volatile compound that is released into the atmosphere by transpiration for dispersal (Limmer and Burken 2016); phytoextraction is a technology used when plants are able to accumulate toxic compounds in the harvestable parts such as stems and leaves (Burges et al. 2017); rhizoremediation consists of the elimination of the contaminant by the microorganisms in the rhizosphere (Kuiper et al. 2004a); and phytoremediation as a strict term refers to the transformation of the contaminant by the metabolism of the plant (Macek et al. 2000).

Because plants are easy to monitor and agricultural technologies are constantly being developed, a priori the easiest way to design a phytoremediation protocol would be to use a plant as the single degradative organism. Many plants are able to transform (but in general not mineralize) some contaminants via their unique secondary metabolism, which is represented by the capacity of members of the cytochrome P450 super family of proteins to activate the contaminant that is subsequently conjugated with glutathione, malonyl, or glucosyl residues. The conjugated compound is then accumulated in vacuoles or in the plant cell wall (Burken 2004). These transformation steps are similar to those which take place in the liver of animals and led to the term “green liver” to designate the capacity of the plant to transform contaminants into less toxic compounds. Moreover, pollutants containing amino or hydroxyl groups can undergo rapid transformation by root-associated or extracellular enzymes (Shaw and Burns 2003).

Unfortunately plants do not possess the extraordinary repertoire of degradative enzymes of microorganisms, and therefore, the utilization of phytoremediation strategies based exclusively on plant activities cannot be used universally.

3 Rhizoremediation

Due to their impressive degradation capacity for xenobiotic compounds, microorganisms have long been studied and assayed in bioremediation treatments. In contrast to the successful isolation of microorganisms with specific biodegradation properties, their reintroduction into soil to remove pollutants has been frequently unsuccessful. In some cases it has been reported that reintroduced microorganisms are unable to establish themselves in the soil. Although knowledge regarding the behavior of microorganisms in the environment is incomplete, it is thought that nutrient limitation, competition with indigenous microbiota, and/or limitations in their reactions under environmental stress conditions may account for the lack of success of (re)introduced microorganisms in contaminated soils. Some of these obstacles can be overcome by using rhizoremediation, a strategy that is based on the “rhizosphere effect” that relies on the plant excreting a number of chemicals that can be used as a carbon, nitrogen, sulfur, or phosphorous source by microorganisms to proliferate and reach high cell densities in the area surrounding the roots of the plant, i.e., the rhizosphere. The advantage of rhizoremediation is that plant roots provide a large surface on which microorganisms can proliferate by creating biofilms. Roots transport microorganisms through the soil both in terms of distribution and depth, and as mentioned above, the roots provide nutrients. Root penetration into the soil also facilitates oxygen exchange and thereby the proliferation of aerobic microorganisms and oxygen availability for the initial attack on many different chemicals in reactions mediated by mono- and dioxygenases.

3.1 Rhizoremediation Requirements

One of the classic papers in the field of “natural” rhizoremediation is the report by Radwan et al. (1995) showing that plants growing in sand contaminated by oil spills after the Gulf War exhibited clean roots due to the removal of oil compounds by microorganisms. After this initial observation, many laboratories started to study rhizoremediation, and although there are not many examples detailing the removal of pollutants from soils under real conditions undertaken via the concept of “designed” rhizoremediation, there have been significant advances in the understanding of the interaction between plants and bacteria during rhizoremediation. For a successful strategy, microorganisms have to be able to proliferate in the root system, and catabolic pathways have to be operative and free of the catabolite repression effect, which is the preferential use by microorganisms of a given carbon or nitrogen source with respect to the target compound (Burken 2004), moreover the contaminant has to be bioavailable (Fig. 1).

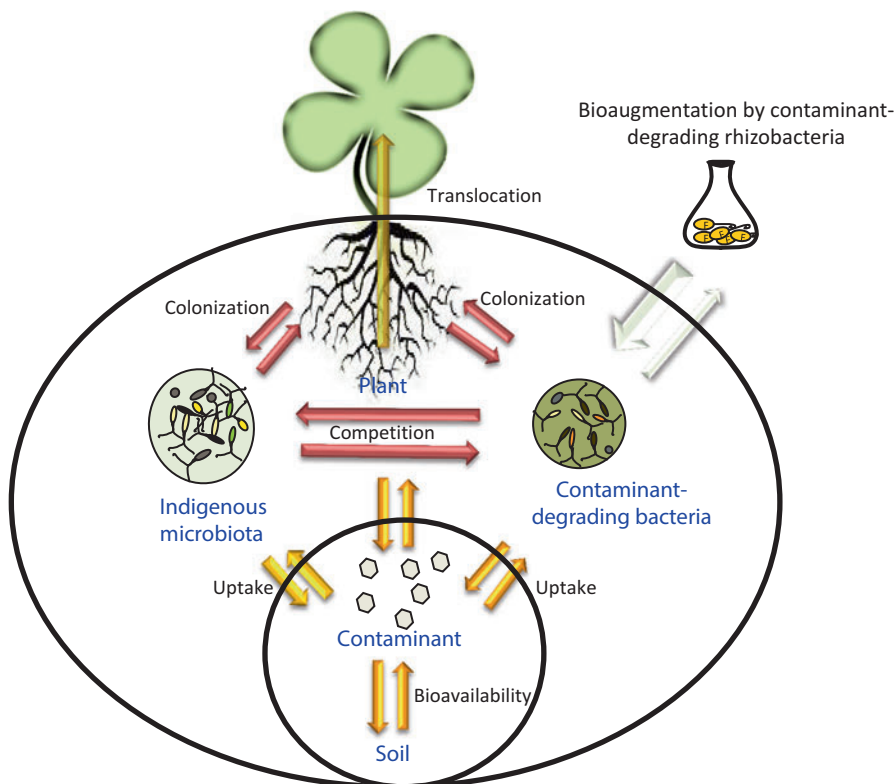


Fig. 1 Interactions influencing rhizoremediation

Rhizoremediation requires the augmentation of the desired bacteria (or consortia) in the laboratory to obtain a sufficient number of degradative bacteria to inoculate the seeds or roots of plants. After growing in the laboratory, the bacteria have to be able to proliferate on the root system under natural conditions (Fig. 1). Early experiments using *in vivo* expression technology (Rainey and Preston 2000; Ramos-González et al. 2005) and microarrays revealed the bacterial genes involved in the adaptation to the rhizosphere (Matilla et al. 2007; Attila et al. 2008). These findings indicated that after growth in the laboratory, bacteria have to reprogramme themselves to be able to live in the rhizosphere. Induction of genes related with changes in the transcriptional pattern of catabolic pathways to enable the utilization of the chemicals secreted by roots as carbon or nitrogen sources, and the induction of bacterial genes involved in oxidative stress are part of the response to the rhizosphere environment. It has also been repeatedly demonstrated that flagellar and chemotaxis genes are involved in root colonization (Lugtenberg et al. 2001; Scharf et al. 2016). These results revealed that the rhizosphere, although nutrient-rich compared with bulk soil, is a nutritionally challenging environment which is stressful to microorganisms due to the formation of reactive oxygen species produced by the respiration of root cells.

Identification of plant microbiomes (Bulgarelli et al. 2012) has confirmed that microbial populations in the rhizosphere depend on plant age, physiological status, and even the cultivar, supporting the idea that plant roots exert a certain selection toward the microorganisms living in their rhizosphere. The idea of specific plant-microbe communication is becoming more popular and, although most of the efforts in rhizoremediation have been focused on degradative bacteria, the utilization of different plants can also influence the outcome of contaminant removal. Microbial elimination of contaminants in the rhizosphere provides a positive effect for the plant as this leads to decreased pollutant concentrations in the area near the roots and thereby improved plant growth in contaminated areas (Rodríguez-Conde et al. 2016). Because of this mutual benefit, it has been proposed that plants can select for specific bacterial genotypes to be present on their roots. Experiments performed by Siciliano et al. (2002) demonstrated that the presence of the alkane monooxygenase gene was more prevalent in endophytic and rhizosphere microbial communities than in bulk soil contaminated with hydrocarbons. However, the results obtained when they studied the prevalence of xylene monooxygenase or naphthalene dioxygenase genes were just the opposite, their presence was higher in bulk soil microbial communities than those near to or on the plant. This effect was also dependent on the plant species used during the rhizoremediation strategy (Siciliano et al. 2003). Therefore, the combined influence of plants and contaminants determines the associated plant microbiota (Fig. 1). The presence of certain contaminants may affect the relative abundance of genotypes able to degrade this particular contaminant (Rodríguez-Conde et al. 2016) or the diversity of rhizosphere or endophytic bacteria (Liu et al. 2017). This has led to the hypothesis that the effectiveness of rhizoremediation strategies also depends on the selection of the best plant-bacteria combination for a specific contaminant (Thijs et al. 2016).

To complicate the general scheme, foreign bacteria also have to be able to compete with indigenous microorganisms suggesting another level of communication; they have to be able to sense their environment (Venturi and Keel 2016; Fig. 1). It is well known that bacteria are able to establish an intraspecific communication using auto-inducers that are synthesized when populations reach a critical density in a process called *quorum sensing*. The *luxI* gene encodes the auto-inducer synthase and the *luxR* codes for an auto-inducer-dependent activator responsible for coordinating the expression of several genes as well as the induction of the *luxI* gene (Stevens and Greenberg 1997; Fuqua and Greenberg 2002). *luxR* and *luxI* are generally located near to each other in the chromosome (Choudhary et al. 2013). However, as more microbial genomic information has become available in databases, it has been observed that many microorganisms encode several *luxR*-type genes that are not in the vicinity of any *luxI* and that there are more *luxR* genes than *luxI*. These genes have been named “orphan-*luxR*” or “*luxR*-solos” (Fuqua 2006). Some of these *luxR*-solos have been involved in intraspecific communication (microbe-microbe interactions) whilst others have been involved in inter-kingdom (plant-microbe) communication (Subramoni and Venturi 2009; Gonzalez and Venturi 2013).

Even if the reintroduced degradative-bacteria are: (i) able to grow with the nutrients available in the rhizosphere; (ii) able to cope with stressful environments; and (iii) able to compete with other microorganisms and to communicate with their environment, all these properties are useless if the degradative genes are not expressed in the rhizosphere. The rationale behind rhizoremediation strategies is that the rhizosphere environment contains additional C-sources that favor microbial growth which consequently accelerates degradation rates (Kuiper et al. 2004a; Lu et al. 2017). However, several reports have indicated that biodegradative genes may be affected by catabolic repression. Catabolic repression consists of the preferential utilization of some carbon sources over others; i.e., the toluene degradation pathway of the pWWO plasmid is repressed in the presence of glucose or succinate (Duetz et al. 1996). Rentz et al. (2004) reported that root exudates from different plants inhibited the phenanthrene-degrading activity of *Pseudomonas putida* ATCC 17484, although these exudates allowed the growth of the strain. Other reports, however, indicate the fortuitous induction of catabolic genes in the rhizosphere (Casavant et al. 2003; Segura et al. 2017), probably as a consequence of the structural similarities between certain plant secondary metabolites and some xenobiotic chemicals (Singer et al. 2003).

3.2 Rhizoremediation Experiments

Different rhizoremediation strategies have been used to demonstrate the utility of this approach for the degradation of contaminants. Initially, bacterial strains that were well characterized for their capacity to degrade a certain contaminant were chosen, but frequently these strains were not well suited for survival in the rhizosphere. This has led to the current tendency to use rhizosphere bacteria. Using a double enrichment approach in which lindane degradation and root proliferation of bacteria were prerequisites for selection, Böltner et al. (2008) isolated *Sphingomonas* strains able to adhere to corn seeds at levels two orders of magnitude higher than the reference strain *Sphingomonas* UT26 and were able to colonize the root at approximately 5×10^8 CFUs (colony forming units) per gram of root. In controlled rhizoremediation experiments, these recently isolated strains were able to degrade 30% of the γ -hexachlorohexane of contaminated soil (0.5 mg per gram of soil) in 25 days. Amongst the disadvantages of this approximation are the lack of information about the degradative abilities of the bacteria and the lack of information about the ability of these bacteria to persist in the specific plant environment. These disadvantages can be alleviated if bacteria are isolated directly from the contaminated soil that has to be cleaned up (Zafra et al. 2017), but this approach requires additional experimentation in order to find the optimal bioaugmentation conditions to ensure that most of the new inoculum contains biodegradative bacteria.

Another approach is the utilization of bacteria that are generally present in contaminated environments. For example, different metagenomic experiments have revealed that in PAHs-contaminated soils, bacteria belonging to the genera *Arthrobacter*, *Rhodobacter*, *Pseudomonas*, *Burkholderia*, *Bacillus*, *Mycobacterium*, or to the *Sphingomonadaecea* family are frequently found to be the most abundant

(Uhlik et al. 2012; Kappell et al. 2014; Rodriguez-Conde et al. 2016). Despite possible biases in the metagenomic analysis, it has been demonstrated that members of these genera and families are good PAH degraders (Lu et al. 2011). It is therefore interesting to test members of these families in rhizoremediation experiments. Utilization of single strains as well as consortia has demonstrated the utility of this approach (Zafra et al. 2017; Rodriguez-Conde et al. 2016).

Finally, the utilization of endophytes in the biodegradation of pollutants is an interesting option that is currently being explored (Afzal et al. 2014). In order to be successful, endophytes with interesting catabolic properties have to be isolated (Taghavi et al. 2009); alternatively, degradative bacteria in the rhizosphere have to be able to transfer interesting genetic determinants to plant endophytes (Mastretta et al. 2006). Natural horizontal transfer of the pTOM plasmid from *Burkholderia cepacia* isolated from yellow lupine, to poplar endophytes that then acquired the capacity to degrade toluene have already been demonstrated (Taghavi et al. 2005). For the successful utilization of this strategy, however, contaminants have to be transported into the plant interior and the transport mechanisms of the contaminants into the root cells have to be explored.

3.3 Monitoring Rhizoremediation

In rhizoremediation there are several parameters that have to be monitored: plant survival and growth, inoculum survival and contaminant elimination. Whilst initially, the number of colony forming units (CFUs) using selective media was almost the only method to follow up microbial survival and rhizosphere colonization, the advances in the utilization of reporter genes, such as the green-fluorescent protein (Gfp) and luciferase, have allowed the simplification of methods to monitor microbial survival in the rhizosphere (Ramos et al. 2000; Buddrus-Schiemann et al. 2010). However, these methods involve the genetic modification of the organisms and therefore, monitorization under natural conditions is mainly achieved by counting cells on selective media.

Contaminant biodegradation monitoring requires the extraction of the contaminant from soil and subsequent determination by analytical methods. These methods (mainly gas chromatography coupled to mass spectrometry [GC-MS] and high-pressure liquid chromatography [HPLC]) require expensive equipment and frequently, analyses of the samples in specialized laboratories. In the last decade, several biosensors (devices based on biological parts that sense a signal and transform it into a quantifiable response) for easy *in situ* detection of contaminants have been developed (Tecon and van der Meer 2008; Segura et al. 2017). However, the toxicity of the contaminant toward the biosensor (if whole cell biosensors are used) and the possibility of synergistic effects with other compounds in the environment can alter the results making biosensors, in general, not as accurate as chemical methods (Diplock et al. 2010). Nevertheless, robust biosensors with high specificity for oil-derived compounds are available (Kohlmeier et al. 2008; Sevilla et al. 2015; Hernández-Sánchez et al. 2016). When chemical methods are used, the total amount of the

contaminant is detected, whereas the biosensors detect the bioavailable fraction; this is important as the bioavailable fraction determines the ecotoxicity of the contaminant (Tecon and van der Meer 2008).

4 Improving Rhizoremediation: Research Needs

One important problem for soil bioremediation, whatever technique is used, 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. Soils with a long history of contamination are generally much less responsive to rhizoremediation than freshly spiked soils (Olson et al. 2007). It has been demonstrated that surfactants, in general, can improve the degradation of the contaminants (Ortega-Calvo et al. 2013). However, as they lead to an increase in the bioavailability of the contaminant, the addition of these compounds may produce negative effects on microbial and plant growth during rhizoremediation. Therefore, rhizoremediation techniques have to be carefully designed to improve the bioavailability of the contaminant without affecting growth. Microbial biosurfactants have been widely studied (Marchant and Banat 2012) and commercial biosurfactants are already available; however, their use in rhizoremediation has not been thoroughly investigated (Liduino et al. 2018). The selection of bacteria which are able to produce biosurfactants in the rhizosphere of plants is an interesting alternative for improving the removal efficiency. In this context, Kuiper et al. (2004b) identified bacteria growing in a PAH contaminated area that produce biosurfactants (putisolvins) that facilitate the solubilization of PAHs and hence biodegradation by microorganisms. This property is also of interest because a number of biodegradative microorganisms exhibit positive chemotaxis towards the pollutants. Therefore, the combined action of biosurfactants and chemotaxis can contribute to bacterial proliferation and to microbial spread in polluted soils, in order to clean larger zones. Besides amino acids, sugars, and other small molecular-sized compounds, plant rhizodeposition including mucilage, high molecular compounds, and other substances could improve the bioavailability of contaminants fostering biodegradation by microbial activity. To the best of our knowledge, no studies about the solubilization of hydrophobic contaminants by plant rhizodeposits have been reported.

Advances in knowledge regarding the molecular interactions between plants and microorganisms, in the expression of catabolic genes in the rhizosphere, and in the selection of the best plant-microbe combinations will have to be converted into field strategies that demonstrate the usefulness of this approach. The release of non-native and recombinant microorganisms into the soil is still a controversial subject and more studies to analyze the impact on indigenous microbial communities are needed.

It is important to know the fate of the contaminants during rhizoremediation as the translocation of contaminants to the aerial part of the plant may facilitate their spread through the trophic chain. Adsorption of the contaminants to the root cell wall and/or transport of the toxic chemical into the plant are research topics that have

received increasing attention in recent years (Dupuy et al. 2016; Cennerazzo et al. 2017). As the economic cost of bioremediation efforts is one of the main aspects that are being considered by contaminant producing industries and it has been demonstrated that the microbial biodegradation activity in soil favors the optimal growth of the plant (Van Dillewijn et al. 2007; Rodriguez-Conde et al. 2016; Vergani et al. 2017), it will be interesting to study the possibility of receiving direct economic benefits through the cultivation of crops while remediation is taking place. The economics of this new perspective and the safety-related issues regarding the possibility of the introduction of contaminants into the food-chain are areas of research that remain unexplored.

Acknowledgments The work by authors was supported by research grants from the Spanish Ministry of Science and Innovation and the Andalusian Regional Government, (Junta de Andalucía). We thank Angela Tate for improving the use of English in the manuscript.

References

- Afzal M, Khan QM, Sessitsch A (2014) Endophytic bacteria: prospects and applications for the phytoremediation of organic pollutants. *Chemosphere* 117:232–342. <https://doi.org/10.1016/j.chemosphere.2014.06.078>
- Attila C, Ueda A, Cirillo SLG, Chen W, Wood TK (2008) *Pseudomonas aeruginosa* PAO1 virulence factors and poplar tree response in the rhizosphere. *Microb Biotechnol* 1:17–29
- Böltner D, Godoy P, Muñoz-Rojas J, Duque E, Moreno-Morillas S, Sánchez L, Ramos JL (2008) Rhizoremediation of lindane by root-colonizing *Sphingomonas*. *Microbial Biotech* 1:87–93
- Buddrus-Schiemann K, Schmid M, Schreiner K, Welzl G, Hartmann A (2010) Root colonization by *Pseudomonas* sp. DSMZ 13134 and impact on the indigenous rhizosphere bacterial community of barley. *Microb Ecol* 60:381–393. <https://doi.org/10.1007/s00248-010-9720-8>
- Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E et al (2012) Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* 488:91–95. <https://doi.org/10.1038/nature11336>
- Burges A, Alkorta I, Epelde L, Garbisu C (2017) From phytoremediation of soil contaminants to phytomanagement of ecosystem services in metal contaminated sites. *Int J Phytoremediation*. <https://doi.org/10.1080/15226514.2017.1365340>
- Burken JG (2004) Uptake and metabolism of organic compounds: green-liver model. In: McCutcheon SD, Schnoor JL (eds) *On phytoremediation*. Wiley, New York, pp 59–84
- Casavant NC, Thompson D, Beattie GA, Phillips GJ, Halverson LJ (2003) Use of a site-specific recombination-based biosensor for detecting bioavailable toluene and related compounds on roots. *Environ Microbiol* 5:238–249
- Cennerazzo J, de Junet A, Audinot J-N, Leyval C (2017) Dynamics of PAHs and derived organic compounds in a soil-plant mesocosm spiked with 13C-phenanthrene. *Chemosphere* 168:1619–1627
- Choudhary KS, Hudaiberdiev S, Gelencser Z, Goncalves Coutinho B, Venturi V, Pongor S (2013) The organization of the quorum sensing luxI/R family genes in *Burkholderia*. *Int J Mol Sci* 14:13727–13747. <https://doi.org/10.3390/ijms140713727>
- Colleran E (1997) Uses of bacteria in bioremediation. In: Sheehan D (ed) *Methods in biotechnology*, vol 2: bioremediation protocols. Humana Press Inc., Totowa
- Diplock EE, Alhadrami HA, Paton GI (2010) Application of microbial bioreporters in environmental microbiology and bioremediation. *Adv Biochem Eng Biotechnol* 118:189–209

- Dos Santos JJ, Maranhão LT (2018) Rhizospheric microorganisms as a solution for the recovery of soils contaminated by petroleum: a review. *J Environ Manage* 210:104–113. <https://doi.org/10.1016/j.jenvman.2018.01.015>
- Duetz WA, Marqués S, Wind B, Ramos JL, van Andel JG (1996) Catabolite repression of the toluene degradation pathway in *Pseudomonas putida* harboring pWW0 under various conditions of nutrient limitation in chemostat culture. *Appl Environ Microbiol* 62:601–606
- Dupuy J, Leglize P, Vincent Q, Zelko I, Mustin C, Ouvrard S, Sterckeman T (2016) Effect and localization of phenanthrene in maize roots. *Chemosphere* 149:130–136
- Fuqua C (2006) The QscR quorum-sensing regulon of *Pseudomonas aeruginosa*: an orphan claims its identity. *J Bacteriol* 188:3169–3171. <https://doi.org/10.1128/JB.188.9.3169-3171.2006>
- Fuqua C, Greenberg EP (2002) Listening in on bacteria: acyl-homoserine lactone signalling. *Nat Rev Mol Cell Biol* 3:685–695. <https://doi.org/10.1038/nrm907>
- Gómez-Sagasti MT, Epelde L, Alkorta I, Garbisu C (2016) Reflections on soil contamination research from a biologist's point of view. *Appl Soil Ecol* 105:207–210. <https://doi.org/10.1016/j.apsoil.2016.04.004>
- Gonzalez JF, Venturi V (2013) A novel widespread interkingdom signaling circuit. *Trends Plant Sci* 18:167–174. <https://doi.org/10.1016/j.tplants.2012.09.007>
- Hernández-Sánchez V, Molina L, Ramos JL, Segura A (2016) New family of biosensors for monitoring BTX in aquatic and edaphic environments. *Microb Biotechnol* 9:858–867. <https://doi.org/10.1111/1751-7915.12394>
- Kappell AD, Wei Y, Newton RJ, Van Nostrand JD, Zhou J, McLellan SL, Hristova KR (2014) The polycyclic aromatic hydrocarbon degradation potential of Gulf of Mexico native coastal microbial communities after the Deepwater Horizon oil spill. *Front Microbiol* 5:205. <https://doi.org/10.3389/fmicb.2014.00205>
- Kohlmeier S, Mancuso M, Deepthike U, Tecon R, van der Meer JR, Harms H, Wells M (2008) Comparison of naphthalene bioavailability determined by whole-cell biosensing and availability determined by extraction with Tenax. *Environ Pollut* 156:803–808. <https://doi.org/10.1016/j.envpol.2008.06.001>
- Kuiper I, Lagendijk EL, Bloembergen GV, Lugtenberg BJJ (2004a) Rhizoremediation: a beneficial plant–microbe interaction. *Mol Plant Microbe Interact* 17:6–15
- Kuiper I, Lagendijk EL, Pickford R, Derrick JP, Lamers GEM, Thomas-Oates JE, Lugtenberg BJJ, Bloembergen GV (2004b) Characterization of two *Pseudomonas putida* lipopeptide biosurfactants, putisolvin I and II, which inhibit biofilm formation and breakdown existing biofilms. *Mol Microbiol* 51:97–113
- Liduino VS, Servulo EFC, Oliveira FJS (2018) Biosurfactant-assisted phytoremediation of multi-contaminated industrial soil using sunflower (*Helianthus annuus* L.). *J Environ Sci Health A Tox Hazard Subst Environ Eng* 1:1–8. <https://doi.org/10.1080/10934529.2018.1429726>
- Limmer M, Burken J (2016) Phytovolatilization of organic contaminants. *Environ Sci Technol* 50:6632–6643. <https://doi.org/10.1021/acs.est.5b04113>
- Liu J, Xiang Y, Zhang Z, Ling W, Gao Y (2017) Inoculation of a phenanthrene-degrading endophytic bacterium reduces the phenanthrene level and alters the bacterial community structure in wheat. *Appl Microbiol Biotechnol* 101:5199–5212. <https://doi.org/10.1007/s00253-017-8247-z>
- Lu XY, Zhang T, Fang HH (2011) Bacteria-mediated PAH degradation in soil and sediment. *Appl Microbiol Biotechnol* 89:1357–1371. <https://doi.org/10.1007/s00253-010-3072-7>
- Lu H, Sun J, Zhu L (2017) The role of artificial root exudate components in facilitating the degradation of pyrene in soil. *Sci Rep* 7:7130. <https://doi.org/10.1038/s41598-017-07413-3>
- Lugtenberg BJ, Dekkers L, Bloembergen GV (2001) Molecular determinants of rhizosphere colonization by *Pseudomonas*. *Annu Rev Phytopathol* 39:461–490
- Macek T, Mackova M, Kas J (2000) Exploitation of plants for the removal of organics in environmental remediation. *Biotechnol Adv* 18:23–34
- Marchant R, Banat IM (2012) Biosurfactants: a sustainable replacement for chemical surfactants? *Biotechnol Lett* 34:1597–1605. <https://doi.org/10.1007/s10529-012-0956-x>

- Mastretta C, Barac T, Vangronsveld J, Newman L, Taghavi S, Van der Lelie D (2006) Endophytic bacteria and their potential application to improve the phytoremediation of contaminated environments. *Biotechnol Genet Eng Rev* 23:175–207
- Matilla MA, Espinosa-Urgel M, Rodríguez-Hervá JJ, Ramos JL, Ramos-González MI (2007) Genomic analysis reveals the major driving forces of bacterial life in the rhizosphere. *Genome Biol* 8:R179
- Olson PE, Castro A, Joern M, DuTeau NM, Pilon SEAH, Reardon KF (2007) Comparison of plant families in a greenhouse phytoremediation study on an aged polycyclic aromatic hydrocarbon contaminated soil. *J Environ Qual* 36:1461–1469
- Ortega-Calvo JJ, Tejeda-Agredano MC, Jimenez-Sanchez C, Congiu E, Sungthong R, Niqui-Arroyo JL, Cantos M (2013) Is it possible to increase bioavailability but not environmental risk of PAHs in bioremediation? *J Hazard Mater* 261:733–745. <https://doi.org/10.1016/j.jhazmat.2013.03.042>
- Radwan S, Sorkhoh N, El-Nemr I (1995) Oil biodegradation around roots. *Nature* 376:302
- Rainey PB, Preston GM (2000) In vivo expression technology strategies: valuable tools for biotechnology. *Curr Opin Biotechnol* 11:440–444
- Ramos C, Molina L, Mølbak L, Ramos JL, Molin S (2000) A bioluminescent derivative of *Pseudomonas putida* KT2440 for deliberate release into the environment. *FEMS Microbiol Ecol* 34:91–102
- Ramos-González MI, Campos MJ, Ramos JL (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
- Rentz JA, Alvarez PJJ, Schnoor JL (2004) Repression of *Pseudomonas putida* phenanthrene-degrading activity by plant root extracts and exudates. *Environ Microbiol* 6:574–583
- Rodríguez-Conde S, Molina L, González P, García-Puente A, Segura A (2016) Degradation of phenanthrene by *Novosphingobium* sp. HS2a improved plant growth in PAHs-contaminated environments. *Appl Microbiol Biotechnol* 100:10627–10636
- Salt DE, Smith RD, Raskin I (1998) Phytoremediation. *Annu Rev Plant Physiol Plant Mol Biol* 49:643–668
- Scharf BE, Hynes MF, Alexandre GM (2016) Chemotaxis signaling systems in model beneficial plant-bacteria associations. *Plant Mol Biol* 90:549–559. <https://doi.org/10.1007/s11103-016-0432-4>
- Segura A, Ramos JL (2013) Plant–bacteria interactions in the removal of pollutants. *Curr Opin Biotechnol* 24:467–473. <https://doi.org/10.1016/j.copbio.2012.09.011>
- Segura A, Hernández-Sánchez V, Marqués S, Molina L (2017) Insights in the regulation of the degradation of PAHs in *Novosphingobium* sp. HR1a and utilization of this regulatory system as a tool for the detection of PAHs. *Sci Total Environ* 590–591:381–393. <https://doi.org/10.1016/j.scitotenv.2017.02.180>
- Sevilla E, Yuste L, Rojo F (2015) Marine hydrocarbonoclastic bacteria as whole-cell biosensors for n-alkanes. *Microb Biotechnol* 8:693–706. <https://doi.org/10.1111/1751-7915.12286>
- Shaw LJ, Burns RG (2003) Biodegradation of organic pollutants in the rhizosphere. *Adv Appl Microbiol* 53:1–60
- Siciliano SD, Fortin N, Mihoc A, Wisse G, Labelle X, Beaumier D, Ouellette D, Roy R, Whyte LG, Banks MK, Schwab P, Lee K, Greer CW (2002) Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. *Appl Environ Microbiol* 67:2469–2475
- Siciliano SD, Germida JJ, Banks K, Greer CW (2003) Changes in microbial community composition and function during a polyaromatic hydrocarbon phytoremediation field trial. *Appl Environ Microbiol* 69:483–489
- Singer AC, Crowley DE, Thompson IP (2003) Secondary plant metabolites in phytoremediation and biotransformation. *Trends Biotechnol* 21:123–130
- Stevens AM, Greenberg EP (1997) Quorum sensing in *Vibrio fischeri*: essential elements for activation of the luminescence genes. *J Bacteriol* 179:557–562

- Subramoni S, Venturi V (2009) LuxR-family ‘solos’: bachelor sensors/regulators of signaling molecules. *Microbiology* 155:1377–1385. <https://doi.org/10.1099/mic.0.026849-0>
- Taghavi S, Barac T, Greenberg B, Borremans B, Vangronsveld J, van der Lelie D (2005) Horizontal gene transfer to endogenous endophytic bacteria from poplar improves phytoremediation of toluene. *Appl Environ Microbiol* 71:8500–8505
- Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N, Barac T, Vangronsveld J, van der Lelie D (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. *Appl Environ Microbiol* 75: 748–757. <https://doi.org/10.1128/AEM.02239-08>
- Tecon R, Van der Meer JR (2008) Bacterial biosensors for measuring availability of environmental pollutants. *Sensors* 8:4062–4080
- Thijs S, Sillen W, Rineau F, Weyens N, Vangronsveld J (2016) Towards an enhanced understanding of plant–microbiome interactions to improve phytoremediation: engineering the metaorganism. *Front Microbiol* 7:341. <https://doi.org/10.3389/fmicb.2016.00341>
- Uhlik O, Wald J, Strejcek M, Musilova L, Ridl J, Hroudova M, Vlcek C, Cardenas E, Mackova M, Macek T (2012) Identification of bacteria utilizing biphenyl, benzoate, and naphthalene in long-term contaminated soil. *PLoS One* 7(7):e40653. <https://doi.org/10.1371/journal.pone.0040653>
- Van Dillewijn P, Caballero A, Paz JA, González-Pérez MM, Oliva JM, Ramos JL (2007) Bioremediation of 2,4,6-trinitrotoluene under field conditions. *Environ Sci Technol* 41:1378–1383
- Venturi V, Keel C (2016) Signaling in the rhizosphere. *Trends Plant Sci* 21:187–198. <https://doi.org/10.1016/j.tplants.2016.01.005>
- Vergani L, Mapelli F, Zanardini E, Terzaghi E, Di Guardo A, Morosini C, Raspa G, Borin S (2017) Phyto-rhizoremediation of polychlorinated biphenyl contaminated soils: an outlook on plant-microbe beneficial interactions. *Sci Total Environ* 575:1395–1406. <https://doi.org/10.1016/j.scitotenv.2016.09.218>
- Zafra G, Absalón ÁE, Anducho-Reyes MÁ, Fernandez FJ, Cortés-Espinosa DV (2017) Construction of PAH-degrading mixed microbial consortia by induced selection in soil. *Chemosphere* 172:120–126. <https://doi.org/10.1016/j.chemosphere.2016.12.038>