Rhizospheric plant-microbe interactions that enhance the remediation of contaminated soils

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The soil, as any other ecosystem, is an important habitat to thousands of organisms associated like a wide variety of fungi, actinobacteria, algae, protozoa and different types of bacteria that vary in physiology. These microorganisms can occur in association to clay particles or organic matter and in the rhizosphere of plants essential to their metabolism in synergism with plant roots; this term can be defined as the three units interacting: the plant, the soil and the microorganisms.

This manuscript reviews some of the important beneficial plant–microbe interactions that promote plant health and development, particularly those relationships between plants and plant growth-promoting rhizobacteria, plant endophytic bacteria and mycorrhizal fungi; in a way that these microorganisms increase the availability of contaminants and help plants to the extraction and removal of inorganic and organic compounds.

Keywords phytoremediation; pollutants; rhizosphere; plant growth-promoting rhizobacteria; endophytes; mycorrhizal fungi

1. Rhizosphere: a particularly microhabitat for microorganisms

The soil, as any other ecosystem, is an important habitat to thousands of organisms associate, among the microscopic ones, there is a wide variety of fungi, actinobacteria, algae, protozoa and bacteria [1]. The microorganisms can occur in association to clay particles or organic matter, in the rhizosphere of plants and in small colonies in the pores among the particles [2,3].

Microbial–plant interactions were largely investigated; however, these studies aimed mainly the plant– pathogen interactions. Only ten years ago, the ecology of microbes in the rhizosphere was focused to many kinds of decontamination processes.

The rhizosphere defined by Hiltner as the volume of soil that is influenced by the roots of plants [4,5]. and according to Lynch [6], this term can be defined as the three units interacting: the plant, the soil and the microorganisms. The composition of rhizosphere structure is highly orientated by the type of plant, quantity and composition of root exudates and different root zones [7,8].

The root-associated microorganisms establish a synergism with plant roots and can help the plant to absorb nutrients improving plant performance and consequently the quality of soils [9-11].

1.1 Soil bacteria: benefic microorganisms for plants

Bacteria may interact with and affect the growth of plants in a variety of ways. Some bacteria are phytopathogenic and actively inhibit plant growth; others (plant growth-promoting bacteria) can facilitate the growth of plants using a wide range of different mechanisms; and there are a large number of soil bacteria that do not appear to affect the growth of plants one way or the other, although this may vary as a function of a range of different soil conditions [12].

With the discovery of a number of soil microorganisms that are capable of degrading xenobiotic chemicals including herbicides, pesticides, solvents, and other organic compounds; microbial degradation might provide a reasonable and effective means of disposing of toxic chemical wastes.

Due to the sensitivity and the sequestration ability of the microbial communities to heavy metals, microbes have been used for bioremediation [13-15]; although microbial communities in metal-polluted bulk soils have been studied, there is little information on the composition of microbial community in the plant rhizosphere growing in soils highly polluted with heavy metals [16].

2. Phytoremediation a especially kind of bioremediation

Bioremediation is a technique that uses living organisms to manage or remediate polluted soils, is an emerging technology and defined as the elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes into their less toxic forms [18,19], can be applied *in situ* or *ex situ*, depending on the site that they will be applied.

Soils in the same way as aquatic environments are the target of thousands of contaminants that vary in composition and in concentration. These contaminants enter the system as a result of a wide range of actions such as intentional applications, inadequate residue disposal, accidental wastes and inappropriate use [20]. The pollution by inorganic compounds as nitrates, phosphates and perchlorates [21]; explosives such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) [22]; monoaromatic hydrocarbons like benzene, toluene, ethylbenzene and xylene (known as BTEX) [23]; polycyclic aromatic hydrocarbons [24]; a range of herbicides such as diuron, linuron and chlorotoluron [25] and by heavy metals [26].

In the case of soil remediation, there are several factors that should be considered, such as soil characteristics; type and concentration of contaminants [27,28].

The possibility of using plants in environmental remediation is an emerging alternative to the restoration of contaminated sites [29-36]. However, this methodology requires an understanding of the intrinsic factors that contribute to its success.

Phytoremediation has become an attractive topic of research and development. Plant-assisted bioremediation, or phytoremediation, is commonly defined as the use of green or higher terrestrial plants for treating chemically or radioactively polluted soils.

Phytoremediation can be classified according to the method and/or nature of the contaminant [37-41] so; there are several names as follows:

a) Phytoextraction: a removal process taking advantage of the unusual ability of some plants to (hyper-) absorbing and accumulating or translocating metals or/metalloids to the shoots.

b) Phytostabilization (and immobilisation): a containment process using plants often in combination with soil additives to assist plant installation to mechanically stabilizing the site and reducing pollutant transfer to other ecosystem compartments and the food chain; the organic or inorganic compound can be incorporated to the lignin or to soil humus.

c) phytostimulation: the growing root promotes the development of rhizosphere microorganisms capable of degrading the contaminant, using exudates as carbon source.

d) Phytovolatilisation/rhizovolatilisation: removal processes employing metabolic capabilities of plants and associated rhizosphere microorganisms to transform pollutants into volatile compounds that are released to the atmosphere. Some ions of elements of sub-groups II, V and VI of the periodic table like mercury, selenium and arsenic are absorbed by root, then converted into less toxic forms and released.

e) phytodegradation: organic contaminants are degraded or mineralized by specific enzyme activity.

f) rhizofiltration : use terrestrial plants to absorb, concentrate and/or precipitate contaminants in the aqueous system.

3. Assisted phytoremedation by microorganisms: rhizoremediation

It is interesting to apply plants combined to some microorganisms to increase the efficiency of contaminants extraction; such technique is called rhizoremediation [42]. Chaudhry et al. [43] report that the use of this beneficial association can work in a way that the microorganisms raise the availability of the compounds and the plants help in the extraction and removal of such compounds. It is positive for both sides, once the plants supply nutrients for microorganisms, which in turn, grow and multiply, increasing the capacity of degradation by plants or increasing the phytotoxicity of the contaminated soil [44]; however, little is known about this synergism between plants and microorganisms and the factors that generate the answers [19].Some of the particular microorganisms that participated in the microbe-assisted phytoremediation, are describe in the next sections.

3.1 Plant growth-promoting bacteria and rhizoremediation

Plant growth-promoting bacteria may facilitate plant growth either indirectly or directly [45]. The ability of plant growth-promoting bacteria to act as biocontrol agents against phytopathogens and thus indirectly stimulate plant growth may result from any one of a variety of mechanisms including antibiotic production, depletion of iron from the rhizosphere, induced systemic resistance, production of fungal cell wall lysing enzymes, and competition for binding sites on the root [45-47]. Plant growth promoting bacteria can directly facilitate plant growth. They may fix atmospheric nitrogen and supply it to plants; synthesize siderophores which can sequester iron from the soil and provide it to plant cells which can take up the bacterial siderophore–iron complex and synthesize phytohormones such as auxins, cytokinins and gibberelins, which can act to enhance various stages of plant growth; solubilize minerals such as phosphorus, making them more readily available for plant growth.

3.1.1 Rhizoremediation of organic contaminants by PGPR's

Although PGPR was first used for prompting the plant growth and for the biocontrol of plant diseases, much attention has recently been paid on bioremediation with PGPR [48-50]. In contrast with inorganic compounds, microorganisms can degrade and even mineralize organic compounds in association with plants [51]. Bacteria capable of degrading certain kind of organic pollutant, such as polychlorinated biphenyls (PCBs) have been isolated from a range of sites and the pathways and encoding genes have also been well studied [52]. But most of these bacteria cannot survive in the near-starvation conditions found in soils, including the rhizosphere [17,53,54].

3.1.2 Rhizoremediation of metals facilitated by PGPR's

Scientists sometimes attempt to increase metal bioavailability through the addition of various chelating agents, a strategy that often works on a small scale in the laboratory but is much less effective in the field. A large number of plants have been tested for their ability to take up high levels of metals and then translocate those metals from roots to leaves and shoots, however, many so-called hyperaccumulating plants do not produce sufficient biomass to make this process efficient in the field [55]. The use of soil bacteria (often plant growth-promoting bacteria) as adjuncts in metal phytoremediation can significantly facilitate the growth of plants in the presence of high (and otherwise inhibitory) levels of metals [17,56].

3.3 Endophytic microorganisms: a particularly type of microorganisms associated to plants applied to rhizoremediation

Endophytic bacteria can be defined as bacteria colonizing the internal tissues of plants (an intimate niche) without causing symptoms of infection or negative effects on their host [57,58]. With the exception of seed endophytes, the primary site where endophytes gain entry into plants is via the roots. Several microscopic studies confirm this route of colonization [59,60]. Once inside the plant, endophytes either reside in specific plant tissues like the root cortex or the xylem or colonize the plant systematically by transport through the vascular system or the apoplast [61,62]. Endophytic bacteria have been isolated from a variety of healthy plant species ranging from herbaceous crop plants [58, 63-65] and different grass species [66,67] to woody tree species [68-70]. In general, Pseudomonaceae, Burkholderiaceae and Enterobacteriaceae are among the most common genera of cultivable endophytic species found [71].

Additionally to their beneficial effects on plant growth, endophytes have considerable biotechnological potential to improve the applicability and efficiency of phytoremediation [72].

Using both cultivation and cultivation-independent techniques, Idris et al. [73] investigated the endophytes and rhizobacteria with *Thlaspi goesingense,* a hyperaccumulator of Nickel. Generally, most of the endophytes were cultivation-independent and tolerated higher concentration of Ni than rhizosphere bacteria. Although this system is promising in heavy metal remediation, the mechanisms by which endophytes promote metal accumulation are not well understood yet and the application of cultivation-independent microbe is very difficult [72].

3.4 Mycorrhiza and plants: a new advance strategy named mycorrhizoremediation

Besides symbiotic bacteria, Fungi can also be used in phytoremediation technology, like arbuscular mycorrhizae, establishing symbiotic relationships with 80–90% land plants [54,74,75].

Mycorrhizae can efficiently explore the soil volume and, due to their small diameter, microsites that are not accessible for plant roots. They can further modify pollutant bioavailablity in several ways, including competition with roots and other microorganisms for water and pollutant uptake, protection of roots from direct interaction with the pollutant via formation of the ectomycorrhizal sheath, and impeded pollutant transport through increased soil hydrophobicity [76]. Ectomycorrhizal associations can display considerable resistance against toxicity in soil polluted with metals [76,77] and organic compounds such as m-toluate [78], petroleum [79], or polycyclic aromatic hydrocarbons [80]. The structure of the fungal sheath and the density and surface area of the mycelium are likely to be important characteristics determining the efficiency of an ectomycorrhizal association to withstand metal toxicity and to protect the host plant from pollutant contact [81].

In addition to their protective role, mycorrhizae may contribute to the resistance of plant–microbial associations through enhanced degradation of organic pollutants in the mycorrhizosphere [76], thus lowering the bioavailable concentration of the pollutant in soil.

In some cases, arbuscular mycorrhizal fungi have been shown to increase uptake of metals [82-84] and arsenic [85,86] in plants but other studies showed no effect or decreased concentrations in plant tissues [87,88]. The contrasting results are difficult to evaluate and may be partly due to different experimental settings, e.g. greenhouse [85,86] versus field studies [87,88] as in the case of arsenic uptake in *Pteris vittata* inoculated with arbuscular mycorhizal fungi [89].

4. Importance of genetically-engineered rhizobacteria to rhizoremediation

Many bacteria in the rhizosphere show only limited ability in degrading organic pollutants. With the development of molecular biology, the genetically-engineered rhizobacteria with the contaminant-degrading gene are constructed to conduct the bioremediation in rhizosphere [56].

Examples about the molecular mechanisms involved in the degradation for some pollutants such as trichloroethylene (TCE) and PCBs has been studied [52,54].

For heavy metals, Sriprang et al. [90] introduced *Arabidopsis thaliana* gene for phytochelatin synthase (PCS; PCSAt) into *Mesorhizobium huakuii* subsp. *rengei* strain B3 and then established the symbiosis between *M. huakuii* subsp. *rengei* strain B3 and *Astragalus sinicus*. The gene was expressed to produce phytochelatins and accumulate Cd^{2+}, under the control of bacteroid-specific promoter, the nifH gene [91].

To select a suitable strain for gene recombination and inoculation into the rhizosphere, there are two criteria that has been recommended: first, the strain should be stable after cloning and the target gene should have a high expression, second, the strain should be tolerant or insensitive to the contaminant; and third, some strains can survive only in several specific plant rhizosphere [92].

5. References

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