International Journal for Multidisciplinary Research (IJFMR)

E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Synergetic Action of Rhizobiome in Bioremidiation of Organic Pollutants

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ABSTRACT

Pollution can be remediated by physical, chemical, and biological means. However, bioremediation has become increasingly popular as it possesses the biochemical and ecological capacity to degrade organic pollutants and making useful for rehabilitating contaminated soils in a comparatively safer and sustainable way. The rhizobiome is the microbial community nitrogen-fixing densely populating in the immediate environment of plant root system. More specifically the rhizobiome are widely distributed in the soil and root ecosystems. The synergistic action of multiple rhizobial strains enhances both plant growth and remediation of pollutants. With ample amount of nitrogen supply it reduces the need for fertilizers and stimulates the survival of other biodegrading bacteria and thereby lowers the concentration of pollutants. This review of recent advances on nitrogen fixing rhizobiome using biochemical and molecular mechanisms provides a new avenue for the rehabilitation of contaminated soils.

Keywords: Bioremediation, endosphere, heavy metals, nitrogen-fixation, organic pollutants, rhizosphere, rhizospheric degraders

1. Introduction:

The rhizobiome is the complex microbial community in the soil that thrives on plant root exudates and may colonize the root surface or infiltrate the root cortex. This community can increase the root surface area by inducing hairy root proliferation. It may also increase the solubility of metals in the rhizosphere and promote the uptake of soluble metals by the plant. The bacterial rhizobiome, a kind of specialized microorganisms that colonize the plant rhizosphere and endosphere, makes an important contribution to Phytoremidiation.

Rhizobiales, belonging to the alphaproteobacteria, are Gram-negative bacteria of agronomic importance because some species form nitrogen-fixing symbiotic relationships with leguminous plants (Sato et al., 2005). This activity of nitrogen fixation reduces the need of fertilizers in plants growth. Hydrogen H_2) thus liberated as a by-product of the symbiotic nitrogen fixation process has recently been revealed to be a common element with novel bioactive properties that enhances plant tolerance to abiotic factors (i.e., oxidative stress and heavy metal toxicity;(Cui et al., 2013; Jin etal., 2013). Many reports have noted that some leguminous species are heavy-metal resistant and can significantly promote the dissipation of organic pollutants [i.e., polychlorinated biphenyls PCBs), polycyclic aromatic hydrocarbons PAHs), and amide herbicides; (Fan et al., 2008; Hamdi et al., 2012; Carrasco-Gil et al., 2013; Li et al., 2013). Approximately 30% of the agricultural land is estimated to be degraded or contaminated, hreatening agricultural productions. (Alloway and Trevors, 2013; Valentin et al., 2013). In addition to contemporary pollutants such as heavy metals, hydrocarbons, and pesticides, a new generation of persistent organic pollutants POPs) such as polybrominated diphenyl ethers (PBDEs), polychlorinated naphthalenes PCNs), and perfluorooctanoic acid (PFOA) require urgent attention (Lohmann et al., 2007).

Although physico-chemical treatments (i.e., physical removal of contaminated soils, chemical extraction, and the application of chemical reagents) are still the most effective strategies to rapidly remediate heavily polluted sites, they are usually energy-intensive and intrusive for the environment (Segura and Ramos, 2013). In contrast, the less energy demanding bioremediation techniques utilizes living organisms to clean up or stabilize inorganic/organic contaminants from the environment. Therefore, phytoremediation is an environmentally friendly and cost effective approach that provides intangible benefits for the soil ecosystem, including soil carbon sequestration, soil quality improvement, biomass and biofuel production, and biodiversity maintenance (Rajkumar et al., 2012).

The symbiosis between microorganisms and plants has been employed for the elimination of environmental contaminants to achieve high effectiveness and ecological sustainability. The effectiveness of phyto- or microbial-remediation is dependent on: (i) soil physio-chemical properties, such as pH, nutrient/organic matter content, soil surface properties, soil texture and bulk densities that influence plant-soil-water relationships and nutrient availability; (ii) toxicity or bioavailability of the targeted contaminants that reduce the productivity of the impacted soils, the biomass of plants and the degradative ability of microorganisms; (iii) plant species and traits; and (iv) the diversity and richness of the indigenous soil microbial communities or flora (Segura et al., 2009). However, these limitations can be addressed by using molecular tools and through the exploitation of the chemical interactions between the plants and resistant rhizospheric microbes which can enhance plant growth, decrease metal phytotoxicity, and affect metal translocation and accumulation in plants (Li et al., 2012).

2. Role of Rhizobiome in Degradation of Organic Pollutants:

Polycyclic aromatic hydrocarbons, a class of hazardous chemicals consisting of two or more fused benzene rings in various structural configurations, are listed as priority toxic pollutants by the U.S. Environmental Protection Agency due to their carcinogenicity, mutagenicity, and toxicity. (Poonthrigpun et al., 2006), (Ahmad et al., 1997) first isolated and characterized a variety of strains of Rhizobium meliloti in soils contaminated with aromatic/ chloroaromatic hydrocarbons. They also found that the rhizobial population was composed of several phenotypicaliy and genetically distinct strains and that all were effective in symbiotic N_2-fixation. Several strains in the genera *Agrobacterium, Rhizobium, Sinorhizobium,* and *Bradyrhizobium* have proven capacity to thrive in presence of PAHs, PCBs, aromatic heterocycles (i.e., pyridine), or other toxic organic compounds (Keum et al., 2006; Poonthrigpun et al., 2006; Tu et al., 2011.(Hussien et al. 1974) first isolated 22 strains of Rhizobium capable of degrading phenolic compounds (i.e., catechol, protocatechuic acid, p-hydroxybenzoic acid, and salicylic acid). Among them, *Rhizobium sp.* and *R. phaseoli* dissimilated phydroxybenzoate to salicylate and then to gentisic acid before oxidation. Catechol and protocatechuic acid were also directly cleaved by these species, whereas *R. japonicum* converted catechin to protocatechuic acid (Muthukumar et al., 1982).

Acenaphthylene and phenanthrene are ubiquitous PAHs in the environment. Acenaphthylene (600 mg liter1) can be totally degraded by *Rhizobium sp.* strain CU-A1 within three days through the naphthalene-1, 8- dicarboxylic acid metabolism pathway (Poonthrigpun et al., 2006). *Sinorhizobium sp.* C4 can utilize phenanthrene as a sole carbon source, and 16 intermediate metabolites involved in this degradation pathway have been identified. Polychlorinated biphenyls are a class of POPs differing in the number of chlorine atoms (1-10) attached to their biphenyl rings (Passatore et al., 2014). Damaj and Ahmad., (1996) revealed that rhizobia could act as a promising candidate for PCB degradation. Tu et al. (2011) demonstrated that *Sinorhizobium meliloti* ACCC17519 degraded more than 70% of 2,4,4'-TCB (PCB28), which was more efficient than the reported performance of other rhizobial strains. In experiments under aerobic conditions, 2-hydroxy-6-oxo-6-phenylhex-2,4- dienoic acid (HOPDA), the meta cleavage product in the classic PCBs-degradative pathway, was identified using GC-MS as the principal intermediate during the biotransformation of 2,4,4'-TCB by *S. meliloti*.

3. Mechanisms involved in Rhizobiome mediated degradation of Organic Pollutants:

Rhizobiales have shown adapted to recalcitrant pollutants, may be due to (i)development of enzymatic profiles essential for the degradation of toxic pollutants alter continuous exposure. (Zinjarde et al., 2014). (ii) Secretion of some aromatic acids and hydroaromatic biosynthetic intermediates (i.e., quinate and shikimate) commonly found within plants and in the rhizosphere could also support the growth of diverse rhizobial species (Parke et al., 1985) as genes for the catabolism of these compounds have been widely found in the genomes of rhizobiales and are conserved among different members. Expression of these genes plays crucial role in the decontamination and recycling of pollutants by rhizobiome in environment revitalization.

The microbial PCB-degradation system includes two major metabolic steps: (i) anaerobic reductive dechlorination, where PCBs are transformed into less chlorinated congeners; and (ii) aerobic breakdown of the biphenyl structure in lower-halogenated congeners (containing less than five chlorines), resulting in chloro-HOPDA (2-hydroxy-6-oxo-6- phenylhexa-2,4-dienoate), and the production of chlorobenzoic acid, ring opening, and potentially complete mineralization (Passatore et al., 2014). Aerobic rhizobial degradation of PCBs typically proceeds via the oxidative biphenyl pathway encoded by the bph genes that include a multi-component dioxygenase (bph A, E, F, and G), a dehydrogenase (bph B), a second dioxygenase (bph C), and a hydrolase (bph D) in other bacteria. Researchers first found that genomic DNAs from *Rhizobium and Bradyrhizobium* hybridized strongly with the *Comamonas testosteroni*-derived bphABC gene probe, indicating the presence of a similar oxidative degradation system in rhizobia (Ahmad et al., 1997). Xu et al. (2010) and Tu et al. (2011) showed that *R. meliloti* and *S. meliloti* could utilize 2,4,4'-TCB (PCB 28) as a sole carbon and energy source under aerobic conditions, and HOPDA has been identified as the main intermediate during the biotransformation of 2,4,4'-TCB by *S. meliloti*. However, little is known about the dehalogenases responsible for the reductive dechlorination of PCBs in the aerobic rhizobiastrains (Aken et al., 2010).

Most dehalogenaseproducing bacteria are anaerobic species and contain more than one dehaiogenase. The degradation pathways of different rhizobial species vary. Muthukumar et al. (1982) reported that p-hydroxybenzoate was metabolized to protocatechuate that in turn was cut by protocatechuate 3, 4-dioxygenase via the ortho pathway in R. leguminosarum, R. phaseoli, and R. trifolii, whereas R. japonicum degraded p-hydroxybenzoate to catechol that was cut by catechol 1,2-dioxygenase. Moreover, the regulatory mechanisms for rhizobial bioremediation are related to various rhizobial catabolism pathways, thereby indicating that further investigations are needed. For example, the presence of glutamate favored the degradation of p-hydroxybenzoic and salicylic acids but had little effect on catechol during the rhizobial degradation of aromatic compounds (Hussien et al., 1974).

Researchers have also proposed that although some rhizobial species carry DNA sequences that are homologous to degradative genes, they may not be involved in the degradation process and may instead function in some other capacity. Sato et al. 2005) showed that M. loti MAFF303099 and *Bradyrhizobium* *japonicum* USDA110 possessed dehalogenase-like opening reading frame fragments (ORFs) in their genomes and produced functional haloalkane dehalogenases, but they did not function as halogenated compound degraders. Other researchers have reported that some strains of rhizobia (i.e., *S. meliloti*) did not possess genes for the bioremediation of pollutants (Chen et al., 2005). These outcomes could be due to the inefficient induction of degradative genes limited by plant exudates, O_2-tension, cell density signals, and other environmental factors, the genetic background of the rhizobia, the interdependent regulation of the genes, the flux of metabolic intermediates, or the presence of their end products and non-specific transformation products.

4. Plant- Rhizobiome Assisted-remediation of Organic pollutants:

Bacterial bioremediation in the field is often affected by a variety of factors, including microbial competition, fluctuating environmental conditions, and limited nutrients (Chen et al., 2005). Thus, the selection of effective contaminant-degrading bacteria alone is not enough to ensure optimal remediation. Moreover, phytoremediation is often regarded as slow and incomplete due to limitations in plant-metabolic capacities, rooting depths, and the seasonality of plant growth (Abhilash et al., 2012). It has also been proposed that the storage and accumulation of organic pollutants (or their metabolites) and toxic metals in plant tissues reduces plant survival and results in atmospheric contamination via volatilization through the leaves. Taking advantage of microbe-plant cross-talk offers a low-input biotechnology for ecosystem revitalization in toxic and nutrient-limited environments i.e bioaugmentation;(Abhilash et al., 2012). The inclusion of microorganisms interacting with plants will address the weaknesses of the two individual systems. Legumes are considered to be pioneer plants for phytoremediation (Hao et al., 2014). The huge variety in the metabolic pathways employed microbes makes them valuable tools to ass phytoremediation. The endophytic rhizobia, acting as 'microbial logistics,' break down contaminants that have accumulated nodules, greatly reducing phytovolatilization, facilitating phytoremediation in the rhizosphere and other environments.(Sun et al 2011b) showed that the accumulation of PCE was higher in nodules than any other part of the alfalfa plant, indicating that high concentrate of PCBs accumulated in alfalfa nodules Furthermore,(Li et al. 2013) demonstrate that Rhizobium was effective in removing PCE when inoculated with alfalfa in pot experiment Plants can enhance or stabilize rhizobiol degradation and the biotransformation of vario pollutants, which offers many advantages (i) stimulation of legume growth conferred by plant growth-promoting traits of rhizob including nitrogen fixation, phosphor solubilization, phytohormone synthess siderophore release, and the production of ACC deaminase and volatile compounds (i.e acetoin and 2, 3-butanediol); (ii) phytostabilization and phytoextraction of heavy metals by the combined actions of the plan and microbes; (iii) the immobilization contaminants and increasing soil organic content via root exudates; (iv) the provision additional nitrogenous compounds to the sol thereby improving soil fertility and supporting biological growth; and v) the modification of structure and diversity of microflora [ie rhizobacteria and arbuscular mycorrhiza fungi(AMF)], which help immobilize metals, promote microbial degradation, and enhance the growthand phyto-stabilization of the legumes. (deBashan et al., 2011) The phytoremediation of organic contaminants mainly occurs by three mechanisms: phytoextraction, phytodegradation, and phytovolatilization. These three mechanisms demand a high biomass of plants and high availability of pollutants, both of which can be achieved by the N-fixation process and the secretions of rhizobiome. For example, the collaboration between Medicago sativa (alfalfa) and S. meliloti for the removal of aromatic pollutants has been extensively studied. (Mehmannavaza etal. 2002) reported that a combined treatment (*S. meliloti* A-025 and afalfa grown together) was the most effective for PCB biotransformation after 44 days. However, after 270 days' alfalfa grown alone became the most effective treatment, hereas *S. meliloti* alone was the least effective. Field experiments have shown that PCB removal from rhizosphere soil was clearly enhanced in alfalfa simultaneously inoculated with R. meliloti at 90 days after planting. (Xu etal.2008) Similarly, in a pot study found that planting alfalfa inoculated with R. meliloti significantly lowered the initial soil PAH concentrations by 51.4% compared with unplanted control soil.(Teng etal. 2011) Synergistic Interactions with Other Microbes Inoculation of multiple beneficial microbes often provides complementary and additive benefits to plants, revealing the compatibility, and synergy between distinct mutualists (Larimer et al., 2012). The synergistic promotion of plant biomass and activities of indigenous microbial species caused by dual colonization of rhizobia and other microbes could be a potent tool to further intensify bioremediation efficiency. For example, arbuscular mycorrhizal fungus (AMF) could form extended mycelial networks that not only provide organic nitrogen to host plants but also possess the catabolic capacity to remove organic pollutants (Harms et al., 2011). Teng et al. (2010) reported that the co-inoculation of Rhizobium sp. and AMF enhanced the removal rate of PCBs. This could be due to their combined contribution to plant growth and development by improving nutrient conditions and the additive degradative ability of contaminants conferred by the two cleaners (Harms et al., 2011). The positive synergism of these interactions could be limited by plant species and soil conditions. Additionally, combined approaches using both bio stimulation (with exogenous carbon sources) and bio augmentation may be necessary to sustain the timely and effective in situ microbial biodegradation of pollutants (Andeer et al., 2013).

5. Conclusion

Rhizobiome possess the biochemical and ecological capacity to degrade environmental organic chemicals and to. decrease the risk associated with metals and metalloids in contaminated sites. Rhizobiomeassisted phytoremediation provides further environmental and economic benefits for bioremediation. The exploitation of microbemicrobe or plant-microbe interactions between intra-species and inter-species communication in the rhizosphere could represent more integrative approaches to further facilitatebioremediation. However, taking into account of considerable complexity of the soil environment, the determination of the best method to establish appropriate numbers of foreign pollutantdegrading bacteria in the contaminated sites and to ensure that multiple degraders co-work during the entire rehabilitation process requires further studies (Segura and Ramos, 2013). Therefore, the involvement of metabolic enzymes in the potential degradation of organic compounds in rhizobiome requires further studyof genetic and molecular influences of bioremediation strategy. The successful execution of this versatile bioremediation strategy also requires a thorough understanding of the factors regulating the growth, metabolism, and functions of rhizobiomes and indigenous microbial communities at contaminated sites.

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