

# Interactions among Rhizosphere Microorganisms, Mechanisms and Potential Application in Phytoremediation

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**ABSTRACT:** Numerous bacteria, archaea, algae and fungi, accumulated in the root systems of plants, constitute complex plant rhizosphere microbial communities. They have been proved to emerge striking importance in the communication between plant and environment, and shows promising in environmental governance. In this background, this paper briefly introduces the basic interaction mechanisms of rhizosphere microbial community, and further discusses the applications and prospects of this kind of community interaction in environmental bioremediation. On this basis, an idea of constructing a microbe-loading platform based on synthetic biology and its applications in comprehensive ecological remediation is advanced.

## 1. INTRODUCTION

With the growth of the scale of human economic activities, industrial and agricultural wastes are distributed into the nature due to emission, leakage or diffusion. As they are detrimental to both health and environment, the soil/water contamination is becoming increasingly serious. Millions of species of microbes are widely distributed in the biosphere, forming diverse communities and microbiotas which are diverse in many facets. With high surface to volume ratio, high metabolic and reproductive rate and strong adaptability, they become biological resources with great potential for bioremediation. As is usually mentioned, creatures almost never exist in isolation. In these consortia, they form relatively stable associations with elaborate interaction mechanisms. Researches have indicated that these complex inter- or intra- specific interacting mechanisms consist of antagonism [1], metabolic dependency [2], resource competitions [3], biofilm-mediated interactions [4], predation [5] and so on. Because these complex mechanisms provide huge operating space for the performance and regulation of physiological functions of natural and artificial microbial communities, microbial communities and mechanisms of their internal interactions are becoming a hot spot in modern microbiology, and the relationship between different microorganisms is being gradually examined from a systematic perspective.

For all plants that have roots, the root system is their main channel of interacting with the non-atmospheric environment (soil or water). Thus, the rhizosphere embodies an intricate and dynamic biogeochemical and ecological zone, then microbiomes in it are able to exert enormous influence on processes like material exchanges in environmental improvement [6]. The results of

identifying rhizosphere microbial networks associated with wild oat (*Avena fatua*) reveal that they are more complicated than those in soils, which imply that rhizosphere has better potential for building interactions and sharing niches of microbes than soil [7]. Besides, the relative stability of genetic composition of rhizosphere microbial community in a certain spatial range around individuals of a certain plant species implies the fact that the companionship of a particular microbiota may have increased the advantage of certain plant species in natural selection, though this stability may be affected by classifications of land uses and tree species [8]. These characteristics provides the rhizosphere microbial community the scope to apply in the new access of environmental bioremediation.

The purpose of this paper is to briefly summarize several representative mechanisms of microbial interaction in plant rhizosphere microbial community, the application examples and risks of rhizosphere microbial community in environmental bioremediation, which would provide important enlightenment for adjusting and optimizing microbial interaction in microbial community so as to improve the remediation efficiency of microbial community in ecosystem restoring.

## 2. FRONTIERS OF RHIZOSPHERE MICROBIAL COMMUNITY STUDIES

Based on the stable inheritance of plant symbiont, the analysis of the structural composition of rhizosphere microbial communities becomes more valuable. However, because most microorganisms in soil remains unculturable at present, classifying characters like composition and structure of rhizosphere microbial consortia is supposed to rely on the progress in omics analysis techniques and big-data biology [9]. Through the technical means above,

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researchers have gained deeper and more systematic knowledge of the characteristics of rhizosphere microbial consortia of several plant species. For instance, *Arabidopsis* (*Arabidopsis thaliana*) [10] and rice (*Oryza sativa*) [11] have been systematically observed in laboratory and farmland environments. However, due to the existence of species specificity mentioned above, the analysis of rhizosphere microbial communities of a certain plant species can reveal nothing of the general patterns of rhizosphere microbial interactions but some guiding significance for the follow-up research and production improvement.

Another line of research focuses on the concept of “hub” microbes in the community. In a study, such microbes in rhizosphere microbial communities of south beech forest are classified through the next-generation sequencing (Illumina) and systematic biology analysis. They acted as “hubs” directly controlled by many abiotic characteristics and host genomes. Hence, they play a great role in organizing microbial community network, which far exceeds the proportion of their biomass in the community [12]. The study of hub microbes helps to open some new entry points for grasping the overall patterns and functions of complex microbial communities systematically.

### **3. INTERACTION MECHANISM OF RHIZOSPHERE MICROORGANISMS**

The interaction between microorganisms is an important guarantee for the microbial community to establish and maintain its structure, functions and homeostasis. It provides channels for the direct or indirect influence, including metabolic regulation, quorum sensing, biofilm formation, etc., between microbe individuals, whether their species are the same or not, in the consortia. The same is definitely true in the rhizosphere too [13]. In recent years, the mechanisms of rhizosphere microbial interactions documented so far mainly include:

#### **3.1. Metabolic coupling and competition**

Metabolic cross-feeding is a key process applied by many kinds of microbes in order to compensate for deficiencies in metabolic pathways and reduce metabolic burden in the biosynthesis of molecules (like amino acids), complete electron transfer and eliminate toxic substances [14], thus extending their ecological niche. Some studies have suggested that there are bound to be similar (even the same) metabolic pathways among different kinds of microorganisms in the microbial community, and when metabolites can be secreted into the environment, some microbial species that have lost the common metabolic pathway by adaptive gene loss are preserved in natural selection because they can save more resources, although this makes them more dependent on other microbes in the community that have metabolic pathways that they lack. On the whole, under natural selection, the microbes in the microbial community may show a tendency to shift from synthesizing metabolites needed by themselves to synthesizing a wide variety of common metabolites and

maintaining metabolic mutualisms by trading them [15]. It should be pointed out that although the metabolite exchange may reduce the fitness of a single species of microorganism, but for complex communities, their ability to resist fluctuating environments may increase, such as resistance to pernicious biotic and abiotic stress. The mechanism of metabolic coupling and complementarity can also be used for competition and inhibition among microorganisms as the target of some “foxy” microbes.

#### **3.2. Signal molecules mediated microbial interaction**

The fundamental of this interaction mechanism is the sensation of signal molecules secreted by other microorganisms. The difference between this interaction mechanism and metabolic coupling mechanism is that effector molecules in this mechanism are only used to transmit signals. It binds to receptors on microbial cells and regulates physiological activities such as metabolism through signal transduction pathways but does not directly participate in metabolism. An important part of this mechanism is quorum sensing, in which signal molecules are released, detected and responded. These molecules (also known as autoinducers) commonly embrace a wide range of compounds like N-acyl-L-homoserine lactones (AHLs), cis-2-dodecenoic acid (BDSF) [16], etc. Several AHLs that Gram-negative bacteria rely on for communication only differ in length and degree of substitution of the acyl side chain, indicating that their species specificity may not be that strong. Consequently, it provides a basis for AHL's usage as a signal molecule for communication between multiple species of Gram-negative bacteria and the subsequent microbial interaction at the molecular level. There is also a quorum sensing system relying on another autoinducer (AI-2, a set of furanosyl boron esters) which is hypothesized having effects on the virulence of some pathogens, yet its possible impact on the interaction of rhizosphere microbes is still waiting examined. Some facts have now shown that communities containing both bacteria and fungi have some quorum sensing effects triggered by volatile compounds, thiamine and glucose as well [17], so it is easy to assume that the sensing mechanisms in rhizosphere consortia have a high degree of diversity.

#### **3.3. Biofilm-mediated interaction**

Biofilms are complex structures constructed by microorganisms embedded in extracellular polymers produced by themselves and are generally supposed to provide a fundamental basis for properties like nutrient capture, pathogenesis and antimicrobial tolerance. Their role in plant root systems have also been gradually studied in recent years. Moreover, biofilms play important roles in roots such as activating the expression of cooperative social phenotypes [18], enhancing the horizontal gene transfer and the host range of mobile genetic elements [19]. In addition, recent studies suggest that interactions among multiple species of microbes have an important influence

on the function of the biofilm in rhizosphere. For example, with the support of ectomycorrhizal fungi (EMF), the swimming motility and the ability of biofilm formation of several bacteria on mycelium of EMF is enhanced. Fungal-bacterial biofilms associated with arbuscular mycorrhizal fungi in rhizosphere have probably demonstrated significant functional role in the enhancement of plants' tolerance to environmental pollutants and detoxification, which provides a remarkable new idea for environment remediation.

Of course, there are still a vast number of interaction mechanisms that have just been preliminarily identified or are even unclear. For example, contact-dependent interactions based on the Type III and Type VI secretion systems, which are crucial for *Pseudomonas fluorescens* in the colonization of rhizosphere and arbuscular mycorrhization [20, 21], and siderophore-mediated microbial interactions (e.g., collaboration or deception) [3] still exists research aspect.

Through the summary of these interaction mechanisms, it can be found that these mechanisms are effective only when the distance between different microbial individuals is close. This sheds a light for the artificial construction of microbial communities for economic production or exploration of properties of microbial communities in the future: narrowing the physical distance between microbes may help to provide a good foundation for microbial contact at the spatial level.

#### **4. APPLICATION AND RISK OF RHIZOSPHERE MICROBIAL INTERACTION IN ECOLOGICAL IMPROVEMENT**

It should be noticed that most attempts to use these interaction mechanisms to put rhizosphere microbial communities into application are concentrated in agriculture until now, such as studying the feasibility of utilizing them as an indicator of crop status and agro-ecosystem stability or improving agricultural soil management strategies, but some researchers have realized the great possibility of the application of interactions in rhizosphere microbial communities in phytoremediation. A recent study by Mei et al. attempts to clarify how rhizosphere microbes affect the migration and detoxification of metal(loid)s like As, Fe or Mn in mangrove. It may provide inspiration for development of heavy metal bioremediation [22].

In fact, the usage of microorganisms for bioremediation in soil and water is a very prevalent used biological treatment strategy now. Many scholars have identified and obtained a great number of species of functional microorganisms which can be used in bioremediation by means like 16S rRNA gene sequencing and gene microarray. Furthermore, it is noted that compared with applying single strand of microbe, the application of microbial consortia in ecosystem restoration will generally achieve positive results due to their diversity in functions boosted by internal interaction mechanisms, so the bioremediation method of multiple bacteria co-treatment, relying on microbial interactions,

has produced more heart-stirring results than single strains of bacteria in fields not only degradation of aromatic compounds, pesticides but also mineland rehabilitation [23].

For the soil or water environment with complex pollutants, the use of multiple functional microbes for comprehensive remediation is generally regarded to be worth considering. However, because the direct target of microbial interaction system is the whole soil or water environment in the process of bioremediation, there are inevitably some problems:

(1) Due to the lack of understanding of universal interaction mechanisms in the microbial community, when dealing with complex pollutants in soil/water environment, it is difficult to determine the relationship of these functional microbes in the artificial microbial community. If there are negative relationships such as competition or inhibition existing among these functional microbes in the community, there is no doubt that the remediation effect would be notably weakened.

(2) Nowadays, most of the widely used microbial interaction systems have poor spatial specificity. When the functional microbiota is spread into the polluted area, microbes in it will gradually disperse in the environment, which will weaken the microbe-microbe interaction, leading to the decline of the adaptability of microbe individuals and the decrease of their activity. Therefore, the treatment effect is decreased and the cost of achieving treatment effect increases. At the same time, because it is difficult for microbes to regroup and be separated from the environment after functioning at present, there are difficulties to completely remove toxic substances for microbial interaction systems in the treatment of hazardous chemical pollution.

(3) Directly spreading the functional microbial community in the polluted environment is inevitably extensive, and the impact on the ecosystem also needs to be observed. Although researchers could reduce safety risks by selecting original strains in the local environment, avoiding mobile genetic vectors and designing functional bacteria with special phenotypes when designing a microbial system, high microbial biomass and high expression of functional genes (mostly exogenous) that the treatment process requires as well as intermediary metabolites produced in the microbial degradation of pollutants which may be more harmful are potential risks that may disrupt the ecosystem [24].

Most of the problems above arise partly from insufficient understanding of the complex interaction mechanisms and relative uncontrollability of the functional microbes. Under this circumstance, a platform based on genetic modified "carrier" microorganisms that could become joints between the plant root system and functional microbes is probably able to enhance the spatial specificity, versatility and recovery rate of the rhizosphere microbial community. This system is perhaps more effective and controllable on promoting the study on the structure and function of rhizosphere microbial communities than approaches applied before.

## 5. CONCLUSION

This paper briefly sums up several representative interaction mechanisms in rhizosphere microorganisms, comprising of metabolic coupling, competition, signal molecules mediated interactions, biofilm-mediated interactions and contact-dependent interactions, and the application examples of these mechanisms, such as heavy metal removal and mineland rehabilitation, in the field of phytoremediation. However, it is undeniable that the scheme of directly applying microorganisms to restoration of soil and water is still challenging because of some technical and environmental risks. Using synthetic biology technology to construct a rhizosphere microbe-loading platform for comprehensive phytoremediation and qualitative, location or even semi-quantitative studies on rhizosphere microbial community in laboratory is worth considering, yet its prospect in specific application remains to be confirmed in future investigations. When constructing such a microbe-loading platform in future, some aspects should be paid attention: stimulating natural conditions, improving efficiency and integration, and subsequent research on microbe-microbe interactions and microbial metabolite production under pollutant stress. The research progress mentioned in this paper offers theoretical support for the construction (based on microbial interaction) of rhizosphere microbe-loading platform for phytoremediation.

## AUTHORS' CONTRIBUTIONS

This paper is independently completed by Yuming Feng.

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