

Combining dgge and quantitative PCR to evaluate the diversity of *pseudomonas spp.* and *fusarium spp.* in the radix *pseudostellariae* rhizosphere under monoculture

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ABSTRACT

Adix pseudostellariae is a perennial herb with significant medical potential that is used as a tonic. Production and quality suffer significantly when this plant is repeatedly monocultured in the same field. In this work, a 3-year field experiment was conducted to determine the growth inhibitory effect brought on by *R. pseudostellariae*'s protracted monoculture. Along a 3-year monoculture gradient, DGGE analysis was utilised to investigate changes in the composition and diversity of soil *Fusarium* and *Pseudomonas* populations. The findings showed that prolonged monoculture considerably increased the diversity of *Fusarium* species while decreasing the variety of *Pseudomonas* species. Although *Pseudomonas spp.* experienced a reduction, quantitative PCR analysis revealed a

considerable increase in *Fusarium oxysporum*. Furthermore, the abundance of hostile *Pseudomonas spp.* with antagonistic potential against *F. oxysporum* decreased significantly in successively monocultured soils. While inhibiting the growth of antagonistic *Pseudomonas sp.* CJ313, a phenolic acid mixture at the same ratio as that found in soil could promote the mycelial and sporular growth of pathogenic *F. oxysporum*. Additionally, *Pseudomonas sp.* CJ313 performed well in plant bioassays, preventing *F. oxysporum* from infecting *R. pseudostellariae*. In conclusion, this study showed that *Fusarium* and *Pseudomonas* populations in the plant rhizosphere might be altered by an extended monoculture of *R. pseudostellariae*, resulting in a relatively low level of antagonistic microorganisms but a comparatively high level of pathogenic bacteria.

Key Words: *Pseudostellariae*; Monocultured; *Pseudostellariae*'s; Antagonistic; Bioassays; Pathogenic

INTRODUCTION

The continuous monoculture problem, sometimes referred to as "replant disease" or "soil sickness," affects up to 70% of therapeutic plants. Many Chinese medicinal herbs, such as *Panax notoginseng*, *Radix pseudostellariae*, and *Rehmannia glutinosa*, are produced with these issues. The *Caryophyllaceae* family includes *R. pseudostellariae*, a perennial tonic herb with exceptional medical significance. Consecutive monocultures of this plant in the same field result in poor plant performance, a significant decline in root quality and yield, and a severe restriction on the production and use of its medicinal plant virtues. Therefore, it is essential to investigate the process causing ongoing monoculture issues with the plant and create efficient *R. pseudostellariae* control methods.

One of the most common, ubiquitous, and significant soil fungi is the *Fusarium* species. It is notorious for infecting a wide variety of host plants and spreading diseases such as vascular wilts, seedling damping off, and stem rots. Similar to this, soil-borne illness caused by *F. oxysporum* in *R. pseudostellariae* fields has been documented; however, other *Fusarium* species are frequently

overlooked. We therefore need more knowledge to elucidate the various functions played by this potentially significant species if we are to realise the full potential of the disease-suppressive microbial community in biological control.

The development of environmentally friendly and ethical agricultural practises for disease prevention has received increased attention in recent years. Exploring the types of microbial diversity in the soil, especially *Pseudomonas*, under various agricultural regimes or seasons has become crucial. Numerous functional groups, including plant pathogens, xenobiotic degraders, and plant growth promoters, were revealed to be present in *Pseudomonas* species. *Pseudomonas* species can also be used to control soil-borne infections like *Fusarium* wilt, wheat disease, and tobacco black rot.

Recent evidence suggests that plant-microbial interactions are increasingly important for both soil quality and plant health. reported that in soil, the bacterial taxon *Desulfotomaculum ruminis* and the fungal taxon *F. oxysporum* can be stimulated by the peanut root exudates, while certain communal bacteria, such as *Gebria glutamica*, *Mitsuaria*

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chitosanitabida, and *Burkholderia*, can be selectively inhibited. It was discovered that after *Rehmannia glutinosa* monoculture, the number of two harmful fungi increased significantly in the rhizosphere. It showed that long-term continuous cropping of black pepper could result in a significant decrease in the amount of soil bacteria, particularly *Pseudomonas* spp., indicating that soil microbes may be in charge of soil health.

Denaturing Gradient Gel Electrophoresis (DGGE) is regarded as a useful method for analysing the diversity and structure of microbial communities directly. The usual approach of measuring *Fusarium* diversity relies on the identification and isolation of strains that were grown on particular media. However, morphologically identifying *Fusarium* species is a difficult and time-consuming task. It outlined a PCR-DGGE technique to identify various *Fusarium* spp. in environmental samples. The technique is based on the precise amplifying and separating of the transcription elongation factor-1 gene. The 16S rDNA gene of the human genome was used to build a primer set (PsR and PsF) that was based on. To quickly examine the diversity within the genus *Pseudomonas*, a semi-nested PCR and DGGE were devised by combining the PsR and PsF primers. As a result, increased focus has been placed on the role of soil microbial ecology in the prevention and management of plant diseases. There haven't been many studies done to determine how *Pseudomonas* and *Fusarium* of *R. pseudostellariae* are related, as well as how to treat infections that affect this plant.

The alterations in *Pseudomonas* and *Fusarium* populations in rhizosphere soil under *R. pseudostellariae* monoculture were examined in this work using DGGE and qPCR techniques. For the investigation of plant-microbe interactions, a number of microorganisms that were intimately related to the problem of extended monoculture were isolated. Our work can provide helpful information on potential native microflora for soil remediation and improvement, as well as an illustration of the effects of ecological environment and root exudates on the selection of soil microbes in rhizosphere soil.