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Response of Rhizosphere bacterial diversity to phytoremediation of Ni contaminated sediments

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ABSTRACT

Phytoremediation was applied to repair heavy metal-polluted sewage river dredged sediments in a coastal city of China. Six types of plants were used: *Zea mays* L., *Lolium multiflorum* Lam., *Medicago sativa* L., *Brassica junce*, *Elsholtzia splenden* and *Festuca arundinacea* Scherb. A relationship between the concentration of heavy metal Ni and the bacteria diversity in the rhizosphere sediment was analyzed based on the PCR–DGGE (polymerase chain reaction-denatured gradient gel electrophoresis). The results indicated that the quantities and types of microorganism in the rhizosphere sediments differed depending on the type of plant. In three types of plants, the phytoremediation efficiency was highest midway through planting, while the repair effect was highest at harvest time for the other three types of plants. The repair effect order of the former three types of plants was *Zea mays* L.> *Lolium* multiflorum Lam.> *Festuca arundinacea* Scherb, and the repair effect order of the other three types of plants was *Brassica junce*> *Medicago sativa* L.> *Elsholtzia splenden*. The bacteria community structure of *Zea mays* L. changes faster than that of the other plants and stabilizes faster when adapted to the rhizosphere environment. Based on the repair effect and the repair time, *Zea mays* L. is the best plant for Ni phytoremediation. During the growth of plants, the change in the DGGE fingerprint of the bacteria diversity in different periods is similar to the change in the concentration of Ni in the rhizosphere soil. The dominant types of rhizosphere bacteria are plant- and growth period-specific. The Shannon index of the same plant for different growth periods was calculated, and the results indicated that the diversity index changes with the repair process.

1. Introduction

During river way management, the treatment of sediment that contains heavy metals and organic pollutants is a sizable problem. Pollutions produced by heavy metals in the sediments of drainage river have become an important research topic in urban river pollution control (Ran et al., 2013). Among the many chemical, physical and biological processes, phytoremediation includes a wide range of hytotechnologies that aim to improve the environmental status of polluted soil. Phytoremediation is a promising technique for large areas of soil where the emphasis lies on the environmental rather than the economic value (Moreno-Jiménez et al., 2011). Phytoremediation techniques aim to optimize the interaction between soil and plants to decontaminate soils and improve the environmental quality of the site. (Rodriguez et al., 2009; Mench et al., 2009). In phytoextraction, exceptional amounts of contaminants are accumulated in the harvestable tissues that are removed from the site, while phytostabilization aims to improve the soil quality and confine the contaminant in a safer form in the soil (Moreno-Jiménez et al., 2012). Many studies have examined the effects of plant growth and management on the total/available content of contaminants in soils and plants (Dickinson et al., 2009; Ernst, 2005; Mendez and Maier, 2008; Mench et al., 2010). Nickel is an essential nutrient element for people, animals and higher plants (Brown et al., 1987). However, excessive nickel in the soil is toxic to plants (Poulik, 1999; Kapustka et al., 2006; Kopittke et al., 2007).

Along with human activities, such as the mass exploitation of minerals and use of resources, the extensive use of pesticide, fertilizer and sludge and sewage water irrigation, increasing amounts of nickel and its compounds enter agricultural soil. Excess nickel in the soil has not only hindered the growth of plants but also caused the accumulation of nickel in plants. This accumulation is potentially harmful to human health when ingested via the food

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chain. Therefore, the study of the chemical behavior of nickel in soil–plant-microbial systems is environmentally significant. Microorganisms in soil are an important component of the ecosystem. They can indicate the pollution of soil ecosystem and can potentially significantly repair the environment (Kelly et al., 2003). Microorganisms participate in almost all biological and chemical reactions in soil, while the microorganisms in the soil are sensitive enough to reflect the changes in the environmental quality and the biological activity in soil (Aon et al., 2001; Sandaa et al., 1999). Polymerase chain reaction (PCR) has been used to detect and characterize bacterial species in environmental samples for a decade (Kuske et al., 2006). Combined with denaturing gradient gel electrophoresis (DGGE), the products of 16srRNA amplified via PCR can be separated, and PCR–DGGE is a useful way to study the diversity of the microbial community in environmental samples.

In this study, the repair effects of different plants on the heavy metal Ni in polluted sediment were compared, and the function of rhizosphere microorganisms in the repair of Ni was examined. The relationship between Ni and the microorganisms was studied in different plants, and the changes in the bacteria diversity during the growth of different plants were monitored using a PCR–DGGE fingerprint.

2. Materials and methods

2.1. Soil characteristics

The tested sediments were sampled from the dredging sewage sludge of the South sewage river in a coastal city of China. Two sampling sites were selected to obtain the sediments. Because the river is responsible for taking up domestic and industrial wastewater along the river, the water quality of the river is worse than class V (GB3838, 2002), and the main pollutants are heavy metals and organic matter. The physical and chemical properties of the sediments from the two sampling sites were monitored, and the results are shown in Table 1. The tested sediments of the two sites were mixed and triturated using a glass mortar pestle. The initial concentrations of heavy metals in the sediment were monitored.

The concentration of Ni in the sediment exceeded the secondary standard of Soil Environmental Quality Standards by 3.51-fold (GB15618, 1995).

2.2. Experimental set up

Six types of plants were chosen: *Zea. mays* L., *Lolium multiflorum* Lam., Medicago. *sativa* L., *junce, Elsholtzia splenden* and *Festuca arundinacea* Scherb.

Sediment was loaded into six PVC boxes (0.7 m × 0.5 m × 0.3 m). Each box was loaded with approximately 20 kg sediment, and a vent was installed at the bottom of the boxes. The water-holding ratio (WHC) of the sediment in the box was adjusted to 30–60% using deionized water. The seeds were soaked in 5% hydrogen peroxide to disinfection for 5 min and washed by distilled water, after that they were placed in culture dish that with two filter paper. The seeds were sowing in drill, and the distance between each seed is 15 cm. Final singling of seedlings were performed 6 days after plant germination. The planting time ranged from April to October. The rhizosphere soil was sampled by shaking off the clod and collecting the soil attached on the root of the plants. Rhizosphere soil was sampled every 30 days to monitor the concentration of heavy metal Ni and the number of bacteria, and three parallel samples were monitored for each sample. The Ni was detected via HCl–HF–$HClO_4$ digestion.

2.3. Enzyme activity assays

The dehydrogenase activity was tested via the reduction of 2,3,5-triphenyltetrazolium chloride (TTC). The released triphenyl formazan (TPF) was extracted with methanol, assayed at 485 nm and expressed as TPF mg/kg^{-1} h^{-1}. The soil urease activity was determined using the indophenol colorimetric method (Tabatabai and Bremner, 1972). The NH_4^+ released via the urease enzymatic hydrolysis of urea was colorimetrically determined at 578 nm and expressed as NH_4–N mg/kg^{-1} h^{-1}. The sucrase activity was measured using the 3,5-dinitrosalicylic acid colorimetric method (Guan, 1986). The sucrase activity is expressed as the quality of glucose (mg g^{-1}) produced per gram soil after being cultured for 24 h.

2.4. Plate counting of soil bacteria population

Nutrient Agar medium was used to culture bacteria. The total numbers of bacteria in the samples were determined by plate counts.

2.5. DNA extraction and PCR–DGGE analysis

DNA was extracted from 0.5 g soil sample fractions using the Soil DNA Kit (Omega Biotek, US) according to the manufacturer's instructions.

The bacterial 16S rRNA genes were amplified using the universal forward primer. EUBr1387: 5'-GCACAAGCGGTGGAG-CATGTGG-3′ and reverse primer EUBrl387: 5′-GCCCGGGAAC GTATTCACCG-3′, and the GC-clamp was connected to primer EUBf933 (Iwamoto et al., 2000). PCR and DGGE were performed according to the method described by Nakatsu et al. (Nakatsu et al., 2000).

2.6. Data analysis

The means, standard deviation and analysis of variance (ANOVA) were determined using the SPSS computer package (PASW Statistics 18.0). The means were compared using paired-sample *t*-tests with a significance level of $p < 0.05$. The data in the figures are the mean values and standard errors of triplicates.

3. Results and discussion

3.1. Concentration change of heavy metal Ni

The concentration of heavy metal Ni and total bacteria number in the rhizosphere sediment were monitored monthly. Fig. 1 shows the changes in heavy metal Ni in the rhizosphere sediment of different plants. There are three types of plants that the best phytoremediation efficiency was maximized 90 days after planting: they are *Z. mays* L., *L. multiflorum* Lam. and *F. arundinacea*

Table 1
Physic-chemical properties of sewage river sediment.

pH	Available nitrogen (mg/kg)	Available phosphorus (mg/kg)	Organic matter (g/kg)	Heavy metal content (mg/kg dry soil)					
				Zn	Ni	Cr	Mn	Cu	Cd
7.47	220.2	29.75	112	1942.7	175.7	622.1	688.0	276.6	13.5