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Impacts of chelate-assisted phytoremediation on microbial community composition in the rhizosphere of a copper accumulator and non-accumulator

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Abstract

Chelate-assisted phytoremediation has been proposed as an effective tool for the extraction of heavy metals from soil by plants. However, side effects of the addition of chelate to soil microbial community are usually neglected. We studied the potential effects of chelate (glucose and citric acid) amendment on phytoextraction of copper and microbial community composition in soil under laboratory conditions. A copper (Cu) accumulator, *Elsholtzia splendens*, and a non-accumulator, *Trifolium repens*, were grown on a sandy loam soil containing 317 mg kg^{-1} Cu. Microbial community compositions were analyzed by using polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE). The results showed that the biomass of *E. splendens* grown with the chelate did not differ from that of the control. Addition of citric acid decreased the biomass of *T. repens* in comparison to that of glucose treatment. Application of glucose or citric acid significantly increased the extractable Cu concentration in planted and unplanted soils. Concentrations of Cu in the shoots of *E. splendens* were 2.6, 1.9 and 2.9 times of those of *T. repens* under no chelate, citric acid and glucose treatments, respectively. PCR–DGGE fingerprint analysis revealed that there were negative correlations between bacteria diversity and NH_4NO_3 extractable Cu under glucose or citric acid treatment. It was indicated the amendment of glucose to the plant *T. repens* increased the bacteria diversity in soil as compared to that in soils of non-chelate treatment. The above results indicated chelate addition facilitated phytoremediation of soil Cu and did not have a negative effect on microbial community.
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Keywords: Copper contaminated soil; PCR–DGGE; *Elsholtzia splendens*; *Trifolium repens*; Chelates; Rhizosphere

1. Introduction

The spread of heavy metals in the terrestrial environment is largely attributed to anthropogenic activities such as field application of sewage sludge, various industrial activities and the disposal of waste

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products (Kovalchuk et al., 2001). Many reports have shown that short-term or long-term exposure to toxic metals results in the reduction of microbial diversity and activities in soil (McGrath et al., 2001; Lasat, 2002). Diversity and activity of microbial communities are important indexes of soil quality. Soil microbes play significant roles in recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals, and the control of plant pests and plant growth (Giller et al., 1998; Filip, 2002; Elsgaard et al., 2001). Alterations in the composition of microbial communities have often been proposed to be an easily and sensitive indicator of anthropogenic effects on soil ecology (Sitaula et al., 1999).

Cu is an essential element; it forms organic complexes and metalloproteins, especially haemoglobin. With its known antifungal and algicidal properties, elevated levels of Cu in soil adversely affect microbially mediated soil processes. In European Union countries the warning and critical limits of Cu in soil are set at 50 and 140 mg kg^{-1}, respectively (Council of the European Communities, 1986). The strict implementation of environmental laws urges the development of cost-effective soil remediation methods. Phytoremediation, the use of plants to remove, stabilize, or detoxify pollutants, provides an effective and in situ alternative method of cleaning up heavy metals from contaminated soils (Baker et al., 1994; Blaylock et al., 1997). It is reported that phytoremediation not only reduces the environmental risk of soil metal contamination, but also increases the activity and diversity of soil microorganism and improves soil quality (Giller et al., 1998; Filip, 2002).

Successful phytoremediation depends mainly on the bioavailability of heavy metals in the soil; however, the availability of heavy metals for plants is usually restricted by the complexation of metals within solid soil fractions. Chelate-assisted phytoremediation, the use of synthetic chelators, e.g. ethylenediaminetetraacetate (EDTA), has been used to artificially enhance heavy metal solubility in soil and thus increase heavy metal phytoavailability. Nevertheless, it is reported that many synthetic chelators capable of inducing phytoextraction might form chemically and microbiologically stable complexes with heavy metals and pose a threat of soil quality and groundwater contamination. So far researches in chelate-assisted phytoremediation are mainly focused on searching new high efficiency chelates (Kos and Lestan, 2003). Little is known about the potential effect of chelator amendment on the microbial activity and community composition in soil during heavy metal chelate-assisted phytoremediation.

In this study we explored two chelators, glucose and citric acid, on the microbial community composition in Cu contaminated soil during phytoremediation. *Elsholtzia splendens*, an indicator of copper mines and widely distributed on Cu mining wastes and Cu-contaminated soils along the middle and lower reaches of the Yangtze River, China (Tang et al., 1999; Lou et al., 2004), was selected in this study. Another plant *Trifolium repens*, which reportedly is sensitive to heavy metals, was also selected in the experiments. Our objective was to compare bacterial community structure in the rhizosphere of the copper accumulator *E. splendens* and non-accumulator *T. repens* during heavy metal chelate-assisted phytoremediation. We also assess the potential effect of chelators on soil microorganism community composition. Soil bacteria community analysis was carried out using activation-independent methods. Partial 16S rDNA genes were amplified from soil bacterial community DNA by PCR, using primers which bind to evolutionarily conserved regions within these genes in the eubacteria. The diversity of PCR-amplified products was transformed to genetic fingerprints using denaturing gradient gel electrophoresis (DGGE) (Muyzer and Andr, 1993; Luo et al., 2003).

2. Materials and methods

2.1. Soil characterization

Soil was collected from the top layer (0–15 cm) of an agriculture field contaminated with copper in Zhujiawu country, Zhejiang Province, China. According to USDA soil taxonomy (USDA, 1988), the Alluvial sandy loam, paddy soil that developed on the river alluvium is fluvaquents. The soil was air-dried and sieved (<0.45 mm) to remove plant materials, soil macrofauna and stones. Total content of Cu in the soil was analyzed with flame atomic absorption spectrophotometry (FAAS) by digesting 100 mg of soil in a mixture of $HF–HClO_4–HNO_3$ (Lu, 1999). Soil organic material, cation exchange capacity (CEC) and

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