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Technical Note

Trichoderma atroviride F6 improves phytoextraction efficiency of mustard (*Brassica juncea* (L.) Coss. var. *foliosa* Bailey) in Cd, Ni contaminated soils

Lixiang Cao [a], Min Jiang [b], Zhirui Zeng [a], Aixue Du [b], Hongming Tan [a], Yuhuan Liu [a,*]

[a] *State Key Laboratory for Biocontrol and Department of Biochemistry, School of Life Sciences, Zhongshan (Sun Yatsen) University, Guangzhou 510275, PR China*
[b] *School of Environmental Science and Engineering, Zhongshan (Sun Yatsen) University, Guangzhou 510275, PR China*

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Abstract

Trichoderma atroviride F6, isolated from decaying feather and resistant to 100 mg l^{-1} Cd^{2+} and 250 mg l^{-1} Ni^{2+}, was applied for rhizoremediation of Cd, Ni and Cd–Ni combination contaminated soils through association with *Brassica juncea* (L.) Coss. var. *foliosa*. The strain significantly alleviated the cellular toxicity of cadmium and nickel to plants. Inoculation of *B. juncea* (L.) Coss. var. *foliosa* with *T. atroviride* F6 resulted a 110%, 40% and 170% increase in fresh weight in Cd, Ni and Cd–Ni contaminated soils, respectively ($P < 0.05$). The translocation factors and metal bioconcentration factors calculated for the inoculated plant were increased compared to the non-inoculated plants. The results indicated that the efficiency of phytoextraction for *B. juncea* (L.) Coss. var. *foliosa* enhanced after inoculating with *T. atroviride* F6. The fungal treated plants grown in Cd–Ni combination contaminated soils showed higher phytoextraction efficiency than those in Cd or Ni contaminated soils. Thus, it is suggested that the fungus *T. atroviride* F6 endowed with organic-degrading capabilities could be exploited for fungi-assisted phytoremediation of mixed organic-metal contaminated soils.

Keywords: *Brassica juncea*; Cd contamination; Ni contamination; Phytoextraction; Rhizoremediation; *Trichoderma atroviride*

1. Introduction

Soil polluted by heavy metals represents an important environmental problem because of their accumulation throughout the food chain leading to serious ecological and heath problems. Conventional remediation methods, such as soil excavation followed by coagulation–filtration or ion exchange are expensive and disruptive to the sites. In situ bioremediation is gaining momentum as a low-cost and effective method for restoration and remediation under many site conditions (Wu et al., 2006).

Phytoremediation, as a cost-effective and environmentally friendly method, is an emerging technology based on the use of plants to clean up polluted sites. One of ideal plant species to remediate is the *Brassica juncea*, a high biomass plant within the Brassicaceae family (Banuelos and Meek, 1990). However, the distribution of hyperaccumulating plants is limited and the hyperaccumulating ability is metal specific. An important contribution to the phytoremediation is ascribed to microbes in the rhizosphere of plants (Kuiper et al., 2004). The use of plants in combination with microbes has the advantage of causing an increase in microbial population and metabolic activities in the rhizosphere. The plant and microbe combination can not only improve the physical and chemical properties of contaminated soil but also increase the contact between the microbes and contaminants in the soil (Kuiper et al.,

* Corresponding author. Tel.: +86 20 84110238; fax: +86 20 84036215.
E-mail addresses: caolx@mail.sysu.edu.cn (L. Cao), lsslyh@mail.sysu.edu.cn (Y. Liu).

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2004). It has been shown that rhizosphere bacteria may play an important role, probably by increasing the availability of heavy metals for plant uptake (Idris et al., 2004; Wu et al., 2006; Zaidi et al., 2006). Anthropogenic soil pollution is usually not limited to a single contaminant and often several metals are present at high concentration in soils. A limited amount of research has been dedicated to describe the behavior of plant-microbe association in these conditions even if these are the most frequent cases in soil restoration. These studies involve serious difficulties as plant heavy metal uptake is subjected to the antagonistic, additive and synergetic effects that heavy metals exert on one another (Marchiol et al., 2004).

Fungi are known to tolerate and detoxify metals by several mechanisms, including valence transformation, extra and intracellular precipitation, and active uptake (Zafar et al., 2007). The high surface area to volume ratio of fungi and their ability to detoxify metals are among the reasons that they are adopted for remediation of contaminated soils (Kapoor et al., 1999). Fungal strains grouped in the genus *Trichoderma* possess a wide spectrum of evolutionary responses that range from very effective soil colonization, with high biodegradation potential, to non-strict plant symbiosis (Harman et al., 2004). Some mycoparasitic *Trichoderma* strains can tolerate more than one kind of metal (Kredics et al., 2001a,b) . It has been demonstrated that *Trichoderma harzianum* strains can detoxify potassium cyanide and promote root growth of arsenic hyperaccumulating fern *Pteris vittata* (Lynch and Moffat, 2005); *T. harzianum* T22 (also known as Rifai 1295-22) mediates growth promotion of crack willow (*Salix fragilis*) saplings in metal-contaminated soil (Adams et al., 2007). The use of *Trichoderma*-plant association may be applicable for remediating sites with multiple contaminants.

The majority of work so far on plant growth promotion with *Trichoderma* has been carried out on annual crops in uncontaminated soil. Hakka mustard (*B. juncea* (L.) Coss. var. *foliosa* Bailey) is one of the main crops cultivated in subtropical regions of China. The potential of *Trichoderma* to stimulate the growth of Hakka mustard in Cd–Ni contaminated soil has so far not been investigated. The experiment reported in this paper was undertaken to study the basic potential of phytoremediation of mustard-*Trichoderma* association in a Cd–Ni contaminated soil.

2. Materials and methods

2.1. Isolation and identification of isolate F6

The fungus F6 was isolated from decaying feather samples by conventional methods. The strain could grow in a potato dextrose agar medium containing 100 mg l^{-1} Cd^{2+} and 250 mg l^{-1} Ni^{2+}. It was identified by morphological characteristics and ITS1–5.8S–ITS2 region sequence analysis. A polymerase chain reaction was performed to amplify the ITS1–5.8S–ITS2 region of the isolate F6. The forward and reverse primers were 5′-CTTGGTCATTTAGAGGAAGTAA-3′ and 5′-TCCTCCGCTTATTGATATGC-3′. The gene was amplified and sequenced as described previously (Cao et al., 2004).

2.2. Mustard seed sterilization and inoculating with strain F6

Seeds of mustard (*B. juncea* (L.) Coss. var. *foliosa* Bailey) were surface-sterilized by submersing them in sodium hypochlorite solution (3% available chlorine) for 20 min; subsequently, the seeds were rinsed in sterile tap water for three times. The seeds were pregerminated on wetted filter paper for 3 d at 30 °C in dark, and then the germinated seeds were inoculated with spores of strain F6. Inoculating was performed by keeping the germinated seeds in spore suspension (about 10^5 spores l^{-1}) for 30 min at 25 °C. The inoculated seedlings were then air-dried for 5 min on pieces of filter papers and used for further experiments.

2.3. Uncontaminated soil

A loamy soil from the campus of Zhongshan University, sieved (2 mm) and mixed with quartz–sand (<2 mm) (soil:sand = 9:1 (v/v)), was used as the tested soil. The soil had a pH 6.8, 14.7% organic matter, and heavy metal concentrations (mg kg^{-1}): total Cd, 3.8, total Ni, 20.9. The soil texture was made of 38% sand, 50% silt, and 12% clay.

2.4. Contaminated soil

The soil/sand mixture was weighed and the field capacity was calculated. $NiCl_2 \cdot 6H_2O$ solution (42.5 mM) and $CdCl_2$ solution (8.9 mM) were slowly added into homogenized soil to avoid the metal solution run off from the soil. Then the soil of the three treatments (250 mg kg^{-1} Ni^{2+}, 100 mg kg^{-1} Cd^{2+}, and 250 mg kg^{-1} Ni^{2+} + 100 mg kg^{-1} Cd^{2+} were added, respectively) was incubated for 2 weeks to stabilize the added metals.

2.5. Pot experiment

Eight pots (20 cm diameter) were washed thoroughly with tap water and 3 kg of soils was put into each pot. Sixteen seedlings inoculated with strain F6 and 16 control seedlings without any inoculation were transplanted into the 8 pots containing the Cd, Ni or Cd–Ni contaminated soils and uncontaminated soils (four plants per pot). After the first pair of true leaves appeared, seedlings were thinned to 2 seedlings per pot. Three replicates per treatment were made totaling 24 experimental units (two plants per unit) (Table 1).

2.6. Growth conditions

These plants (*B. juncea* (L.) Coss. var. *foliosa* Bailey) were grown in a glass greenhouse under natural lighting and day/night temperature of 27/16 °C. Every two days the pots were weighed, and the water content was adjusted