STUDY ON REMEDIATION OF DIXOIN-CONTAMINATED SOIL BY PLANT-MICROBIAL COMBINATIONS

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Abstract

Perennial ryegrass (*Lolium perenne* L.), Italian ryegrass (*Lolium multiflorum* Lam.) and rye (*Secale cereale* L.) were primarily used for promoting remediation of a soil contaminated with 10,000 pg-TEQ/g of dioxins through combination with *Flammulina velutipes*, a white-rot fungus isolated from eatable mushroom and having a strong ability to degrade organic compounds. These plant-microbial combination systems promoted plant growth, increased the number of total bacteria and fungi in the rhizosphere, and enhanced remediation of the dioxin-contaminated soil. These beneficial effects on plant growth, soil microbes and remediation efficiency varied with plant species. Perennial ryegrass showed the greatest dioxin removal rate when combined with *Flammulina velutipes.* The results suggest that combination of white-rot fungi with suitable plant may be a practical choice for promoting remediation of organic contaminated soils.

Introduction

Soil contamination by various organic compounds such as dioxins and agrochemicals has been a worldwide concern, and phytoremediation has received increasing attention in recent decades as a cost-effective and eco-friendly approach for remediation of these contaminants [1, 2]. Phytoremediation is the use of living plants to remove pollutants from the environment or to render them harmless [2]. Phytoremediation to date indicates that it is applicable to a wide range of organic contaminants, such as petroleum hydrocarbons, pesticides, chlorinated solvents, dioxins, and surfactants [3, 4]. On the other hand, in recent decades, lignin degrading white-rot fungi (WRF) have attracted extensive scientific attention as these fungi are able to degrade a wide range of organic pollutants including dioxins. However, so far, little information is available regarding the combination of WRF and plants for promoting remediation of dioxin-contaminated soils. The objective of this study was to develop plant-fungus combination systems for enhancing remediation of dioxin-contaminated soils.

Materials and Methods

The soil samples used in the present study was a loam soil provided by a waste incineration factory in Saitama, Japan, with a dioxin-content over 10,000 pg-TEQ/g. The soil was air-dried, sieved through 2 mm mesh, and mixed thoroughly for use. Three forage grasses, perennial ryegrass (*Lolium perenne* L.), Italian ryegrass (*Lolium multiflorum* Lam.), and rye (*Secale cereale* L.) were selected to use in this study. Germination and growth of the plants were made in 7-cm pots containing 60g (dry weight) of the dioxin-contaminated soils, and then were incubated in a growth chamber. The lignin degradation white-rot fungus, *Flammulina velutipes*, which was isolated from eatable mushroom and having a strong ability to degrade organic compounds [5], was used in this study. *Flammulina velutipes* was cultivated in a liquid medium based on the method described by Chagas and Durrant [6]. Rhizosphere inoculation was made by adding 20ml of the liquid fungal culture to each pot every two weeks. The corresponding controls for each plant species were made by adding autoclaved (121℃ for 20 min) fungal liquid medium. In addition, two other control treatments, soil only and soil with liquid fungal culture were also prepared. At days 40, 80, and 120 after sowing, three pots in each treatment were randomly removed from the chamber for soil and plant analysis. The shoots and roots of the plants were dried at 40 ℃ in a drying oven for measuring dry plant biomass. Microbial number of total bacteria and fungi in the soil was measured using the spread plate method. The microbial number was expressed as the number of colony forming units (cfu/g). Soil subsamples of the three pots in each treatment were thoroughly mixed to form one soil sample for dioxin analysis. 20 g air-dried soil samples were extracted with toluene using Dionex ASE 200. Then, after adding ^{13}C-labeled internal standards, the extracts were cleaned up. The last fraction was concentrated and spiked with two ^{13}C-labeled PCDD/Fs and four ^{13}C–labeled co-PCBs internal standards for HRGC/HRMS analysis.

Results and discussion

Table 1 shows the total biomass and root biomass of the three plant species with or without *Flammulina velutipes* inoculation. As shown, the total biomass and root biomass of the three plant species continuously increased from 40 to 120 days, showing that they could survive in the dioxin-contaminated soil. PG-FV and IG-FV had much larger total biomass and root biomass, compared with their respective non-inoculation controls, whereas RY-FV had slightly higher total biomass and root biomass than RY control. At day 120, PG-FV produced the highest plant and root biomass among all the treatments, with approximately 56% increment over the PG control (Table 1). The results indicated that *Flammulina velutipes* had beneficial effects on the growth of all three plant species, especially for perennial ryegrass and Italian ryegrass.

Table 1. Plant and root biomass of various treatments at different growing period (mg/pot)

Experimental	40 days		80 days		120 days	
series	Plant	Root	Plant	Root	Plant	Root
PG	53.7±6.7	18.0±5.3	635±61	305±42	2300±225	1183±68
PG-FV	97.3±12	34.0±2.0	990±83	500±44	3587±222	1847±175
IG	140±16	49.7±6.7	1515±127	544±35	2403±156	1083±147
IG-FV	203±42	61.0±16	2094±108	885±45	2890±269	1231±37
RY	233±6.7	138±23	1703±127	640±27	2277±176	728±69
RY-FV	268±19	137±15	1773±257	653±40	2596±110	893±60

Data = mean ± SD (n=3). FV: *Flammulina velutipes*; PG: perennial ryegrass; IG: Italian ryegrass; RY: rye.

The soil of the pots for soil only and soil-FV controls had 5.1-5.9×10^5 cfu/g of total soil bacteria and 2.2-3.2 x 10^5 cfu/g of soil fungi, respectively, and no obvious changes were observed during the time course of the experimental period (Table 1). However, much larger number of both total bacteria and fungi in the soil were observed in the planted pots, compared to that in the non-planted pots, and the number generally increased with plant growth. Moreover, in the planted pots, those treated with *Flammulina velutipes* gave a further increase in soil microbial counts. At day 120, the PG-FV and RY-FV pot soil produced the largest number of both the total bacteria and fungi. These results indicated that the three plant species, especially the perennial ryegrass and rye, stimulated the growth of soil microbes. The application of *Flammulina velutipes* to the rhizosphere seems to have accelerated the soil microbial activities. However, such effect by *Flammulina velutipes* on microbial activities was not observed in the non-planted pot soil. The results indicated that the plants and *Flammulina velutipes* coact to promote the microbial growth, and the effectiveness varies with plant species. Our results are comparable with the previous reports showing that the presence of plant roots increases the size and variety of microbial populations in the rhizosphere [3, 8].

Table 2. Microbial population in various treatments (10^6 cfu/g soil)

Experimental	Day 40		Day 80		Day 120	
series	Total bacteria	Fungi	Total bacteria	Fungi	Total bacteria	Fungi
Soil	0.58±0.10	0.22±0.08	0.51±0.15	0.25±0.10	0.58±0.11	0.32±0.04
Soil-FV	0.59±0.10	0.24±0.04	0.52±0.17	0.27±0.08	0.54±0.20	0.24±0.05
PG	9.86±4.9	5.87±2.7	10.7±2.5	7.93±1.4	14.2±6.7	7.17±2.8
PG-FV	21.0±4.4	15.3±4.0	27.7±7.5	21.3±7.8	59.0±5.6	46.7±5.0
IG	5.43±1.5	2.03±1.0	5.53±0.90	2.77±0.35	6.43±1.6	5.01±1.0
IG-FV	15.3±3.2	6.43±1.2	21.7±2.5	12.6±2.6	25.3±2.5	14.0±1.0
RY	1.80±0.22	0.92±0.61	22.3±4.5	8.47±1.6	12.1±2.1	9.33±1.0
RY-FV	14.3±4.2	8.43±0.57	47.0±4.6	28.3±1.5	54.7±5.7	44.7±5.1

Data = mean ± SD (n=3). FV: *Flammulina velutipes*; PG: perennial ryegrass; IG: Italian ryegrass; RY: rye.

Figure 1 shows the removal rates of soil dioxins in the planted pot soil at day 120. Except for the IG, the planted experimental series had the comparable removal rates ranging 10% to 41% compared with the soil-only control, and the PG-FV was found to have the highest removal rate, followed by IG-FV, PG and RY-FV. Among the planted series without *Flammulina velutipes* inoculation, PG had the highest removal rate, followed by RY and IG. Changes in soil dioxin-concentrations in the soil only control, PG and PG-FV at different experimental periods are shown in Figure 2. Compared to the planted series without *Flammulina velutipes* inoculation, the planted series with *Flammulina velutipes* inoculation generally had a higher removal rate at all sampling periods. These results indicated that although all three plant species with *Flammulina velutipes* inoculation led to greater remediation of soil dioxins, the degrees of promotion effects varied among the plant species.

The removal of persistent organic pollutants by phytoremediation of contaminated soils has been widely studied in recent years. The underlying mechanisms are considered to be the rhizosphere biodegradation, plant uptake, photodegradation, volatilization, root adsorption, and incorporation into soil organic materials [8, 9]. In the enhanced removal of dioxins by the plant-microbial combination systems shown in this study, all such mechanisms might be working.

In conclusion, this study showed that planting perennial ryegrass, Italian ryegrass and rye with *Flammulina velutipes* had beneficial effects on promoting the growth of both plant and soil microorganisms, as well as on remediation efficiency of the dioxin-contaminated soil. Especially, the combination of perennial ryegrass and *Flammulina velutipes* was shown to have effect stronger than other combinations. This would suggest that perennial ryegrass and *Flammulina velutipes* combination is possibly a practical measure for enhancing remediation of dioxin-contaminated soils. Our further study will be to understand the mechanisms involved in the efficacy of the plant-microbial combination systems, and to find other plant-microbial combination systems for enhancing remediation of various organic contaminants.

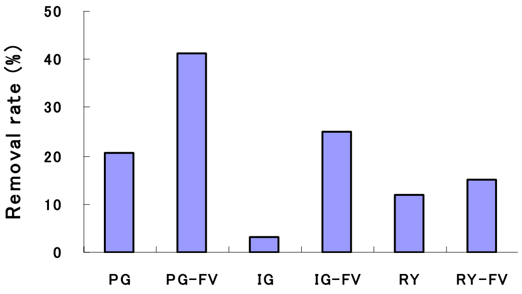

Figure 1. Removal rate of dioxins at day 120 relative to the soil only control

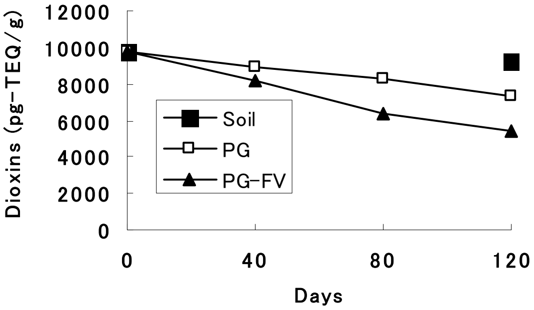

Figure 2. Changes in dioxin concentrations in the soil, PG and PG-FV series with time

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