Effect of Microbial Flocculant on Growth and Cd Accumulation in Different Organs of Rice under Cd stress

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KEYWORDS: Cadmium Stres, Rice Growth, Microbial Flocculant, Cd Accunulation

ABSTRACT: Nine combinations of Cd^{2+} (0,2.5,5mg·L^{-1}) and microbial flocculant (0,10,20mL·L^{-1}) were set, and the hydroponics was adopted in this experiment to explore the effect of microbial flocculant on the rice growth and Cd accumulation under Cd stress, and chlorophyll content, net photosynthetic rate, plant biomass and Cd content in root stem and leaf was determined. The results showed that without Cd stress, adding microbial flocculant could increase the chlorophyll content, promote photosynthesis and plant growth of rice. The inhibition effect of Cd on rice growth was alleviated by adding microbial flocculant. The content of Cd in different organs of rice followed the order: root>stem>dead leaves>fresh leaves. The microbial flocculant inhibited the absorption of Cd2+ by rice root and the transportation of Cd from root to stem and leaf.

INTRODUCTION

Rice is one of the main food crops in the world, but rice also assimilate Cd and accumulate it in the body, so Cd pollution in soil not only reduce yield and deteriorate quality of rice, but also Cd can migrate from food to human body along food chain and injury people's health. At present, the effect of Cd pollution in soil on growth of rice were showed as follows: first, investigation and risk assessment of Cd pollution in the soil. The research of Tang[1] found that the concentration of Cd in paddy soil near the industrial park in Xiangtan was about 1.27-4.22 mg kg^{-1}, belonging to heavy pollution, the potential ecological risk was above 320, suggesting serious ecological harm of the soil; second, Cd distribution in different cultivars, different organs and different growth period of rice. Jiang[2,3] reported that the concentration of Cd in 239 kinds of polished rice was about 0.01-1.98 mg kg^{-1} and there was significant difference among different rice genotypes; third, the effect of environmental factors on the uptake, transportation and metabolism of Cd in rice plant. Cd was transported by apoplastic and symplastic pathways in root, and migrated from root to different organs through transpiration[4,5]. Besides, Eh and pH of soil also influenced the uptake of Cd[6]; fourth, how to control uptake and translocation of Cd, it was reported that the uptake of Cd was inhibited when passivator and organic fertilizer were used in Cd contaminated soil [7,8]. The research of Sun[9] indicated that the concentration of Cd in root, stem, leaf, brown rice and chaff was lower in the treatment with sodium silicate and calcium carbonate than that in the control group in Cd-contaminated soil. Some passivatiors could inhibit the uptake of Cd by rice, but all researches were only in the laboratory because it could cause second pollution for soil, another reason was the high cost.

Bacillus mucilaginosus is common strain of bacteria in soil with strong ability of dissolving potassium, phosphorus and silicon from minerals and fixing nitrogen, so microbial flocculant (MBF) prepared by *B. mucilaginosus* does not cause biological pollution. The preliminary research showed that MBF is environmental-friendly and had a good adsorption for Cd, so MBF was used to control the Cd uptake of rice from culture medium to improve rice quality and ensure food safety.

MATERIALS AND METHODS

Materials

B. mucilaginosus K02 (accession number HM579819) was chosen for the production of MBF. The bacterium was inoculated in a 200 mL medium (1 L medium containing 10.0 g sucrose, 0.3 g yeast extract, 0.5 g $(NH_4)_2SO_4$, 1.0 g K_2HPO_4, 1.0 g $MgSO_4 \cdot 7H_2O$, and 0.5 g $CaCO_3$ in distilled water) and incubated at 30 °C on a shaker (140 rpm) for 3 d. The resultant culture served as a seed culture was inoculated at 10 % volume into 500 mL nitrogen-free medium (1 L medium containing 5 g sucrose, 1.25 g $Na_2HPO_4 \cdot 12H_2O$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCO_3$, 5.0 mg $FeCl_3$, and 50 g illite in distilled water) and incubated at 30°C on a shaker (140 rpm) for 7 d. The culture was used directly as MBF.

The design of experiment

The experiment was carried out in the key laboratory of Environmental Science Department, Tianjin Agricultural University. Rice seeds were disinfected with 75% ethanol for 5 minutes and then sterilized with 0.1% sodium hypochlorite solution for 10minutes. Seeds were germinated in plastic tray in constant temperature incubator with temperature of 35˚C and humidity of 90%, seedlings were raised for 20 days in vermiculite cultures then transplanted to hydroponic pots raised with rice hydroponic formula recommended by the International Rice Research Institute, with N, P and K concentration 40, 10 and 40 $mg \cdot L^{-1}$, respectively. Nutrient solution pH was adjusted with NaOH to about 5.5 every five days after five hours aeration.

A completely randomized design was used, with 3 replicates and 9 treatments of Cd (0,2.5,5$mg \cdot L^{-1}$) and MBF (0,10,20 $mL \cdot L^{-1}$), consistent rice seedlings were selected to make up 27 pots, 6 plants per pot. The 9 treatments are noted as C_0M_0(Cd-0,MBF-0), C_0M_{10} (Cd-0, MBF-10), C_0M_{20} (Cd-0, MBF-10), $C_{2.5}M_0$ (Cd-2.5, MBF-0), $C_{2.5}M_{10}$ (Cd-2.5, MBF-5), $C_{2.5}M_{20}$ (Cd-2.5, MBF-10), C_5M_0 (Cd-5, MBF-0), C_5M_{10} (Cd-5, MBF-5), C_5M_{20} (Cd -5, MBF-10). Chlorophyll content were measured on the14th day, 21stday, 28th day, 35th day, and photosynthetic rate was determined on the 10th day, 21st day and 32nd day after treatments, the biomass ($g \cdot pot^{-1}$) were measured and Cd concentrations of different parts of rice were determined.

Measurement Methods

The chlorophyll content was determined by SPAD-502 chlorophyll meter, net photosynthetic rate (Pn) was monitored by CI-340 photosynthetic apparatus. Cd concentration was determined by atomic absorption spectrometry (TAS-986, China).

RESULTS AND DISCUSSIONS

Chlorophyll content and Pn of rice leaves under different treatments

The chlorophyll content and photosynthetic rate of rice leaves under different treatments was shown in Table 1. Without Cd stress (C_0M_0, C_0M_{10} and C_0M_{20}), the chlorophyll content in the treatment of C_0M_{10} and C_0M_{20} was higher than that in C_0M_0, meaning that with the increase of microbial flocculant dosage, chlorophyll content increased, while, under the condition not adding microbial flocculant, chlorophyll content showed a trend of decrease as the concentration increasing of Cd. Some research has reported that Cd can disturb the activity of chlorophyll synthetase by replacing Mg^{2+} in chlorophyll molecular, resulting in the inhibition of chlorophyll synthesis; at the same time, Cd can increase the activity of chlorophyll decomposition enzyme, thus promoting the decomposition of chlorophyll[10].The microbial flocculant inhibited the toxicity of Cd to chlorophyll synthesis to a certain extent.

As shown in Table 1, without Cd stress, the net photosynthetic rate in the treatment of C_0M_{10} and C_0M_{20} was significantly higher than that in C_0M_0 on the 10th day, and 21st day after treatments， meaning that adding microbial flocculant could increase the photosynthetic rate and rice growth. The photosynthetic rate decreased under Cd stress, but no significant difference was observed between the treatment with and without microbial flocculant (between $C_{2.5}M_{10}$ and $C_{2.5}M_{20}$ with $C_{2.5}M_0$; between $C_{5.0}M_{10}$ and $C_{5.0}M_{20}$ with $C_{5.0}M_0$）at 10 days after treatments. On the 21st day and 32nd day after treatments, adding microbial flocculant showed no significant effect on photosynthetic rate under with or without Cd stress. With the extension of incubation time, the concentrations of microbial flocculant and Cd^{2+} decreased and the harm of Cd to rice reduced.

Table 1 Chlorophyll content and Pn of rice leaves under different treatments

No.	SPAD value				Pn($\mu mol \cdot m^{-2} \cdot s^{-1}$)		
	14d	21d	28d	35d	10d	21d	32d
C_0M_0	34.02	38.83	40.05	39.12	8.87	10.93	4.27
$C_{2.5}M_0$	36.67	36.48	31.38	32.52	7.85	5.80	4.43
$C_{5.0}M_0$	38.52	37.53	35.05	31.40	7.65	6.45	4.5
C_0M_{10}	35.38	39.10	40.65	42.55	10.62	9.60	4.32
$C_{2.5}M_{10}$	35.50	33.65	33.62	39.68	6.91	6.03	4.08
$C_{5.0}M_{10}$	38.83	33.62	36.15	38.22	6.55	6.03	4.77
C_0M_{20}	36.47	39.17	39.38	42.47	9.67	10.22	4.10
$C_{2.5}M_{20}$	34.77	31.97	33.37	33.17	7.20	6.55	4.65
$C_{5.0}M_{20}$	35.57	32.28	35.4	31.92	6.28	5.37	3.48

The biomass of rice plant under different treatments

As shown in Figure 1, without Cd stress, the biomass of rice plant increased with the increasing dosage of microbial flocculant, indicating that adding microbial flocculant stimulated the rice growth. The strain used in the culture of the microbial flocculant in the experiment was *Bacillus mucilaginosus* which can break down silicate minerals, dissolve phosphorus and potassium, fix nitrogen, and so on. At the same time *Bacillus mucilaginosus* can produce some substances during its growth and reproduction period, such as organic acids, amino acids, polysaccharides and hormone, which can promote the growth of plants. Under the condition of Cd 2.5mg/L, the biomass of rice plant was higher in the treatment with microbial flocculant than that without microbial flocculant, indicating that microbial flocculant inhibited the toxicity of Cd to rice growth. When the concentration of Cd was 5.0mg/L, the rice biomass under the treatment adding 10mL/L microbial flocculant was higher than that without microbial flocculant，while, the biomass adding 20mL/L microbial flocculant was slightly lower than that without microbial flocculant， implying that the application rate of microbial flocculant existed a optimal range. The complexation of microbial flocculant with heavy metal inhibited the activity of Cd, but the microbial flocculant existed as colloid state in water since its high molecular weight, so increasing dosage could inhibit plants to absorb nutrient elements.

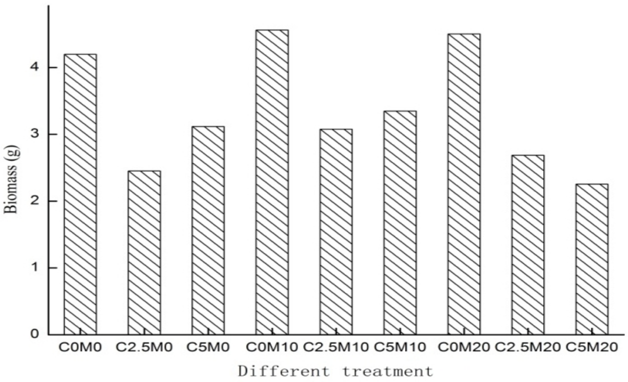

Figure 1. The biomass of rice plant under different treatments

Content of Cd in root, stem, dead leaves and fresh leaves of rice under different treatments

The content of Cd in root, stem, dead leaves and fresh leaves of rice under different treatments was shown in Figure 2. Under no Cd stress, Cd was not detected in rice plant. The content of Cd in different organs of rice followed the order: root>stem>dead leaves>fresh leaves. Under 2.5mg/L Cd^{2+}, significant difference was observed in the content of Cd in root between with and without the microbial flocculant treatment, while, under the treatments with 5mg/L Cd^{2+}， adding microbial flocculant significantly inhibited the transportation of Cd from root to stem and leaf, so the content of Cd in stem and leaf with microbial flocculant was lower than that without microbial flocculant, respectively.

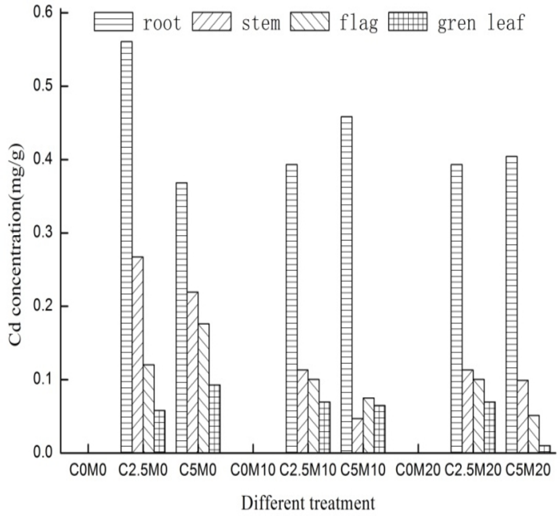

Figure 2. The content of Cd in different organs of rice under different treatments

CONCLUSIONS

Adding microbial flocculant could increase the Chl content, promote photosynthesis and plant growth of rice under no Cd stress. The inhibition effect of Cd on rice growth was alleviated by adding microbial flocculant. The content of Cd in different organs of rice followed the order: root>stem>dead leaves>fresh leaves. The microbial flocculant inhibited the absorption of Cd^{2+} by rice root and the transportation of Cd from root to stem and leaf.

ACKNOWLEDGEMENTS

The study was supported by Sience and Technology Innovation Foundation of Tianjin College Students (201410061090) and Tianjin City High School Science and Technology Fund Planning Project (Grant No. 20130513).

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