Influence of inter-root bacteria on cadmium and arsenic passivation in heavy metal contaminated farmland vegetables

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Abstract. Phytophilic bacteria with phytophilic properties and fixation of soil heavy (analogous) metals can promote plant growth and reduce plant heavy (analogous) metal content. To screen phytoproducing bacteria from the inter-root soil of vegetables grown in heavy (group) metal-contaminated farmland for their ability to reduce As and Cd content in vegetables. Pot experiments were conducted to verify the ability of the functional strains to reduce As and Cd uptake in spinach and green stem cabbage. Functional strains Burkholderia cenocepacia W1-9, Pseudomonas entomophila W1-25, Pseudomonas entomophila W1-51 and Bacillus sp. 51, Pseudomonas entomophila W1-25, Pseudomonas entomophila W1- 51 and Bacillus sp. W2-26. The four test strains had the ability to promote the growth of two leafy vegetables, reduce their uptake and utilisation of Cd and As and stabilise the soil environment, reducing the content of Cd and As in the active state in the soil environment.

Keywords: Leafy vegetable; Rhizosphere soil; Metal(loid)-immobilizing bacteria.

1 Introduction

The development and utilisation of mineral resources have led to the easy enrichment of heavy (analogous) metals in farmland around mining areas, which not only causes deterioration of soil quality and affects crop growth, but also harms health by accumulating in the human body [1, 2]. With the improvement of material level and health consciousness, the per capita intake of vegetables in China is increasing, and the health risk of heavy metals ingested into the human body with vegetables is also increasing day by day [3,4]. Therefore, it is of great significance to fix the heavy (analogue) metals in farmland soil and reduce the content of heavy (analogue) metals in vegetables.

This study used anti-(class) heavy metal plant-promoting bacteria W1-9, W1-25, W1-51, and W2-26 that can reduce the Cd and As content in the active state of the soil, and verified that the four plant-promoting bacteria can reduce the Cd and As content in the edible tissues of green stalks and spinach in a pot experiment, which can provide a

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resource of strains for the remediation of agricultural fields contaminated with heavy (class) metals and for the rehabilitation of agricultural fields contaminated with heavy ((class) metals). class) metal contaminated farmland remediation, and provide reference basis for the structure and function of inter-root soil bacterial community in heavy (class) metal contaminated farmland.

2 Materials and methods

2.1 Potting test

The test soil was taken from 0-25 cm layer of farmland soil in Qixia Mountain Pb-Zn mining area of Nanjing City, Jiangsu Province, with the following heavy metal contents: total Pb 316 mg kg-1, total Cd 4.5 mg kg-1, total Zn 471 mg kg-1, total Cu 83 mg kg-1, and total As 39.4 mg kg-1. The content of organic matter was 14.2 g kg-1, and the pH 6.49. The soil was dried and sieved through 20 mesh sieve. The soil was dried, sieved through a 20-mesh sieve, and divided into 500 g of test soil per pot. Green pedicel and spinach choose the surface without abnormalities, uniform size, full of seeds, aseptic water fully rinsed, put into 75% ethanol disinfection for 3 min, aseptic water rinsing clean alcohol. Sowing sterilised and washed seeds in the test soil, 9 seeds per pot of soil, according to the two kinds of vegetables conventional production methods of management, daily light 12 h. Seeds sprouted eight days later, inter-seedling to each pot to retain 3 seedlings. The treatment groups were inoculated with functional strains W1-9, W1-25, W1-51 and W2-26 (108 cfu mL-1) at 1% concentration. and no inoculum was used as control group. Three replicates were made for each group. After that, the leafy vegetables continued to be cultivated for 30 d. The upper part of the ground and the roots of leafy vegetables were washed with water until there was no obvious soil attached, the roots were soaked in 0.01 M EDTA-2Na solution for 10 min, and the roots were fully rinsed with deionised water, then the washed samples were dried at 50°C until constant weight, and the edible tissues were weighed and measured dry weight, and then the edible tissues were fully pulverised to determine the Cd content of the plants by ICP-OES and As content of the plants by HG-AFS. The Cd content was determined by ICP-OES and the As content by HG-AFS, and the heavy (group) metal content was calculated.

2.2 Analysis of physicochemical properties of soil samples.

Soil samples were air-dried and sieved, 1 g of soil sample was taken in a 10 mL centrifuge tube, 4 mL of DTPA extraction solution was added, shaken for 1.5 h, centrifuged at 10,000 rpm for 15 min, and the Cd content in the supernatant was determined by ICP-OES[5] ; the effective state of As was extracted with 0.05 M (NH4)2SO4, and the As content in the supernatant was determined by HG-AFS[6] , and calculated. Soil effective state heavy (analogue) metal content; soil pH and organic matter content were determined by potassium dichromate volumetric method [7]. Urease activity was determined by colorimetric method, 1M potassium chloride solution extraction, the amount of ammonia generated by the enzymatic reaction using urea as substrate was detected under ultraviolet-visible spectrophotometer (UV2800) at a wavelength of 690 nm, from which the urease content of the soil was calculated[8] .

2.3 Data analysis

Data were statistically analysed using SPSS 19.0, and the test of significant difference between treatments was judged using Duncan's multiple comparisons ($P < 0.05$), and tabulated and plotted using Microsoft Office 2019 software.

3 Results and analyses

3.1 Effect of functional strains on growth and Cd and As content of leafy vegetables

As shown in Table 1, the inoculation treatments had an effect on the edible tissue biomass, Cd and As content of green stem cabbage and spinach. Compared with the non-inoculated control, inoculation with strains W1-9, W1-25, W1-51 and W2-26 significantly increased the edible tissue biomass of green pedicel by 56-91% and significantly reduced the Cd content by 22-42%, strains W1-51 and W2-26 could significantly increase spinach edible tissue biomass (54-57%), while strains W1-9 and W1-25 significantly reduced As and Cd content in spinach edible tissue, respectively. The results indicated that the four test strains had the ability to promote the growth and reduce the uptake of Cd and As in vegetables.

Table 1. The influence of the strains inoculation on the biomass, Cd and As contents of edible tissues of Chinese cabbage and spinach

sample groups	green cabbage			spinach		
	bio-mass(g)	Cd con-tent(mg kg^{-1})	As con-tent(mg kg^{-1})	bio-mass(g)	Cd con-tent(mg kg^{-1})	As con-tent(mg kg^{-1})
CK	1.0 ± 0.2^{c}	4.62 ± 0.66^{a}	0.20 ± 0.02^{ab}	1.09 ± 0.1^{b}	5.40 ± 0.32^{a}	0.33 ± 0.03^{a}
W1-9	1.91 ± 0.2^{a}	2.68 ± 0.06^{c}	0.13 ± 0.02^{c}	1.41 ± 0.3^{ab}	3.62 ± 0.76^{ab}	0.23 ± 0.03^{b}
W1-25	1.56 ± 0.0^{b}	2.88 ± 0.29^{bc}	0.21 ± 0.01^{a}	1.28 ± 0.1^{ab}	3.57 ± 1.09^{b}	0.29 ± 0.01^{ab}
W1-51	1.72 ± 0.1^{b}	3.62 ± 0.17^{b}	0.19 ± 0.02^{ab}	1.68 ± 0.3^{a}	4.43 ± 1.19^{ab}	0.31 ± 0.05^{a}
W2-26	1.69 ± 0.2^{b}	3.27 ± 0.12^{bc}	0.16 ± 0.02^{c}	1.71 ± 0.2^{a}	3.37 ± 0.29^{b}	0.26 ± 0.03^{ab}

Note: Different letters indicate significant differences between the five sample groups ($p<0.05$, Duncan's multiple range test).

As can be seen from Table 2, by comparison with the non-inoculated treatment, inoculated strains W1-9, W1-25, W1-51 and W2-26 in the green pedicel soil showed a significant decrease in pH, a significant reduction in the content of the active state Cd (20-39%), and a significant increase in the content of the urease enzyme (33-100%); W1-9, W1-51, and W2-26, in contrast, significantly reduced the green pedicel soil's active state As (18-35%); W1-9, W1-25, W1-51 and W2-26 similarly significantly reduced Cd (25-39%) and significantly increased urease content (25-50%) in spinach soils while W1-9 significantly reduced As (38%) in spinach soils by significantly lowering the pH of spinach soils. The results showed that the four test strains had the ability to promote the growth of both leafy vegetables, reduce their uptake and utilisation of Cd and As and stabilise the soil environment, reducing the content of Cd and As in the active state in the soil inter-root environment.

Table 2. Effect of strains on soil pH, organic matter, free Cd and As and urease content in green stalk and spinach

sample groups	green cabbage				
	pH	OM	Ex-Cd(mg/kg^{-1})	Ex-As(mg/kg^{-1})	Urease (IU g^{-1})
CK	6.02±0.04^{b}	25.03±0.68^{a}	1.83±0.68^{a}	1.53±0.37^{a}	0.03±0.01^{c}
W1-9	6.61±0.04^{a}	27.19±1.17^{a}	1.12±0.15^{b}	0.81±0.12^{c}	0.05±0.01^{ab}
W1-25	6.87±0.05^{a}	28.70±1.03^{a}	1.26±0.26^{b}	1.51±0.22^{a}	0.06±0.00^{a}
W1-51	6.71±0.13^{a}	26.92±2.33^{a}	1.46±0.11^{b}	1.18±0.15^{ab}	0.04±0.00^{b}
W2-26	6.82±0.07^{a}	27.15±2.71^{a}	1.32±0.17^{b}	1.04±0.13^{b}	0.04±0.00^{b}
sample groups	spinach				
	pH	OM	Ex-Cd(mg/kg^{-1})	Ex-As(mg/kg^{-1})	Urease(IU g^{-1})
CK	6.13±0.10^{b}	24.42±1.18^{a}	1.94±0.15^{a}	1.49±0.15^{a}	0.04±0.01^{c}
W1-9	6.59±0.07^{a}	25.58±2.04^{a}	1.37±0.26^{b}	0.92±0.23^{b}	0.06±0.00^{ab}
W1-25	6.81±0.11^{a}	26.09±1.03^{a}	1.18±0.13^{b}	1.51±0.06^{a}	0.06±0.01^{ab}
W1-51	6.72±0.06^{a}	26.81±0.85^{a}	1.86±0.27^{b}	1.33±0.08^{a}	0.05±0.00^{b}
W2-26	6.72±0.08^{a}	26.33±0.36^{a}	1.46±0.08^{b}	1.29±0.26^{a}	0.05±0.00^{b}

Note: Different letters indicate significant differences between the five sample groups ($p<0.05$, Duncan's multiple range test).

4 Discussion

During plant growth, soil microorganisms not only produce iron carriers and phytohormones (IAA, etc.) to assist growth, but also change the morphology of soil heavy (metalloid) elements and reduce their bioavailability to mitigate the damage to the plants themselves [9, 10]. In the present study, four strains of functional bacteria were isolated and screened from the inter-root soil of two leafy vegetables for their ability to promote plant growth and reduce the heavy metals As and Cd in spinach and green stem cabbage. Strains W1-9 and W2-26 were able to simultaneously reduce the contents of heavy metals Cd and As in the edible tissues of green pedicel, while both of them significantly reduced the contents of As and Cd in the edible tissues of spinach, respectively. In response to the soil physicochemical properties, the four strains of phytoproducing bacteria also had different degrees of positive effects, firstly, all four strains significantly reduced the active state Cd in the soil and substantially increased the urease activity, while strain W1-9 reduced the content of the active state As in the soil of green pedicel and spinach at the same time.

It has been shown that the content of heavy metals in the soil in the effective state was effectively reduced by applying plant-promoting functional bacterial strains [11, 12], which is similar to the results that the content of Cd and As in the soil in the experimental samples in the effective state was significantly reduced by inoculation with the experimental strains.

The effectiveness of heavy metal elements is related to soil pH, the lower the pH value, the higher the proportion of heavy metals in the active state in the soil, and the higher the pH value, the heavy metal elements are precipitated as hydroxides[13] . All four strains were able to significantly increase the pH value of the soil, which in some way favoured the reduction of heavy metals in the soil environment in an active state.All four strains of phytoproducing bacteria were able to significantly increase the urease activity in the inter-root soil. In heavy metal contaminated soils, heavy metal resistant microorganisms are involved in inducing carbonate deposition of heavy metals, which is mainly dependent on the utilisation of urease to urea in the environment to produce CO32- and ammonia, which will result in the generation of carbonate deposits of the metal cations in the soil with carbonate ions, and ultimately reduce the amount of the more biologically harmful active heavy metals in the soil. The content of heavy metals in the active state was found to be effectively reduced by urease-producing bacteria [14], and the treatments inoculated with plant-promoting bacteria in this potting experiment all showed significant increase in urease activity, which further indicates the effectiveness of the function of plant-promoting bacteria to reduce heavy metals in the soil in the active state in this experiment.

5 Conclusions

(1) Four plant-promoting bacterial strains with multiple heavy metal resistance were isolated and screened for their function in increasing the reduction of spinach and green pedlar biomass and decreasing the Cd and As content of edible tissues of both vegetables.

(2) The four functional bacterial strains increased inter-root soil pH and urease activity and reduced the effective state Cd and As content in both vegetables.

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References

1. Jiao W, Chen W, Chang A C, *et al*. (2012) Environmental risks of trace elements associated with long-term phosphate fertilizers applications: a review [J]. *Environmental Pollution*, 168: 44-53.
2. Chen H P, Tang Z, Wang P, *et al*. (2018) Geographical variations of cadmium and arsenic concentrations and arsenic speciation in Chinese rice [J]. *Environmental Pollution*, 238: 482-490.
3. Cen X, Wang D, Sun W, *et al*. (2019) The trends of mortality and years of life lost of cancers in urban and rural areas in China, 1990-2017 [J]. *Cancer medicine*,9: 1562-1571.
4. Zheng R, Chen Z, Cai C, *et al*. (2015) Mitigating heavy metal accumulation into rice (*Oryza sativa* L.) using biochar amendment-a field experiment in Hunan, China [J]. *Environmental Science and Pollution Research*, 22: 11097-11108.
5. REN Zhan-jun, HAO Gui-qi, YIN Chang-tian, et al. (2014) Determination of mercury and arsenic in sludge by microwave digestion-hydride generation atomic fluorescence spectrometry [J]. *Metallurgical Analysis*, 34 (3): 52-56.
6. Glickmann E and Dessaux Y. (1995) A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria [J]. *Applied and Environmental Microbiology*, 61: 793-796.
7. Bao Shi-dan. (2000) Soil agrochemical analysis [M]. *4th Edition. China Agricultural Press*54: 753-776.
8. GUAN SongYin. (1986) Soil enzymes and their research methods [M]. *Beijing. Beijing Agricultural Publishing House*,30 -125.
9. Li Y, Pang H D, He L Y, et al. (2017) Cd immobilization and reduced tissue Cd accumulation of rice (Oryza sativa wuyun-23) in the presence of heavy metal-resistant bacteria [J]. *Ecotoxicology and Environmental Safety*, 138: 56-63.
10. Madhaiyan M, Poonguzhali S and Sa T. (2007) Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (Lycopersicon esculentum L.) [J]. *Chemosphere*, 69: 220-228.
11. Cornu J Y, Parat C, Schneider A. (2009) Cadmium speciation assessed by voltammetry, ion exchange and geochemical calculation in soil solutions collected after soil rewetting[J]. *Chemosphere*, 76(4): 502-508.
12. Jiang C Y, Sheng X F, Qian M, et al. (2008) Isolation and characterization of a heavy metal-resistant Burkholderia sp. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil [J]. *Chemosphere*, 72: 157-164.
13. (2005) Changes of Ni biogeochemistry in the rhizosphere of the hyperaccumulator[J]. *Plant Soil*, 271: 205–218.
14. CAI Hong, WANG Xiaoyu, HAN Hui. (2020) Effects and mechanisms of mineralisation and remediation of Cd and Pb contaminated soil by urease-producing bacteria[J]. *China Environmental Science*, 40(11): 4883-4892.