RESEARCH ARTICLE

# Combination of *Solanum nigrum* and cadmium-resistant bacteria to remediate cadmium-contaminated soil

Xi Luo[1, †], Juhong Zhu[2, †], Bo Ma[1], Juan Hong[1], Weishun Cheng[1, *]

[1]Institute of Environment and Safety, [2]Institute of Crop, Wuhan Academy of Agricultural Science, Wuhan, Hubei, China.

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**The plant-microbe combined remediation technology has become the primary research focus for the treatment of heavy metal-contaminated soils due to the advantages of *in situ* remediation, low cost, environmental friendliness, and high efficiency. To investigate the impact of solanum nigrum and cadmium-resistant strains on cadmium-contaminated soil remediation, this research conducted an outdoor pot experiment with solanum nigrum focusing on assessing the efficacy of these strains in enhancing the growth, cadmium accumulation capacity, and soil remediation efficiency of solanum nigrum. Three cadmium-resistant siderophore-producing strains were screened out from cadmium-contaminated soil, rhizosphere soil of *Solanum nigrum*, and standard strains, which were identified as *Bacillus velezensis*, *Pseudoclavibacter helvolus*, and *Pseudomonas aeruginosa* according to 16S rRNA gene sequences. The results showed that, compared to the non-inoculated control, the shoot dry weights, root dry weights, cadmium total accumulation, shoot cadmium accumulation, transport coefficient, and efficiency of soil remediation of the *Solanum nigrum* treated with strain *Pseudoclavibacter helvolus* + *Pseudomonas aeruginosa* were significantly increased by 31.07%, 75.59%, 30.28%, 31.53%, 39.47%, and 67.83%, respectively. After inoculation with these strains, a significant portion of cadmium in the soil was removed through leaching, while a smaller percentage was absorbed by *Solanum nigrum*. This study was of great significance for the remediation of cadmium-contaminated soil by clarifying the enhanced effect of cadmium-resistant strains from different sources on the hyperaccumulation of cadmium in soil by *Solanum nigrum*.**

**Keywords:** cadmium-polluted soil; cadmium-resistant strains; *Solanum nigrum*; remedying; eluviation.

[*]**Corresponding author:** Weishun Cheng, Institute of Environment and Safety, Wuhan Academy of Agricultural Science, Wuhan, Hubei 430074, China. Email: 405650151@qq.com.

[†]These authors contributed equally to this work.

## Introduction

The problem of heavy metal contamination in soil is becoming more severe due to the use of pesticides and fertilizers, the release of industrial waste gas and wastewater, and the smelting of metal ores [1]. Studies have shown that 19.4% of cultivated land in China exceeds the standard for soil pollution points with cadmium pollution points surpassing the standard at a rate of 7.0%. Cadmium remains the main pollutant affecting the quality of agricultural soil [2, 3]. Therefore, it is essential to explore remediation methods appropriate for soil contamination with cadmium.

The use of plant-microorganism combined technology for remediation of heavy metal-

contaminated soils is a prominent area of study due to its advantages such as *in situ* treatment [4], cost effectiveness [5], environmental friendliness [6], and high efficiency [7]. The mechanism for enhancing phytoremediation by resistant microorganisms primarily involves two main aspects [8, 9]. Firstly, these microorganisms enhance plant nutrition through the synthesis of metal carriers such as siderophores, nitrogen fixation, phosphorus solubilization, and indoleacetic acid production, which in turn boosts plant growth, increases biomass, and improves heavy metal accumulation in above-ground parts. Secondly, resistant microorganisms improve heavy metal bioavailability in soil by acidifying the environment *via* organic acid production, facilitating plant absorption and accumulation of heavy metals. Zhao *et al*. found that inoculating *Bacillus megaterium* into ryegrass rhizosphere soil increased available phosphorus, iron, and cadmium in the soil, as well as ryegrass biomass and cadmium levels in its above-ground parts [10]. Similarly, another research demonstrated that introducing ferriferous bacteria to sweet sorghum roots led to higher iron and phosphorus levels, increased biomass in both above-ground and root parts of sweet sorghum, and elevated cadmium levels in the soil and plant tissues [11]. Additionally, Jiang *et al*. found that the inoculation of *Burkholderia* sp. WS34, known for its deaminase activity, siderophore production, and low levels of IAA, enhanced biomass and cadmium accumulation in Indian mustard and rapeseed [12]. Therefore, cadmium-resistant microorganisms have the potential to promote plant growth and increase cadmium extraction. *Solanum nigrum*, an annual plant belonging to the *Solanaceae* family, displays significant resistance to stress and has a broad distribution. It has shown an impressive ability to accumulate cadmium and has been used in the purification of cadmium-polluted soils. *Solanum nigrum* is known as a cadmium hyperaccumulator. At a concentration of 25 mg/kg, the stem and leaves of *Solanum nigrum* contained 103.8 and 124.6 mg/kg of cadmium, respectively, with a cadmium concentration ratio of 2.68 in the upper part [13]. A previous study showed that the introduction of *Bacillus* into *Solanum nigrum* led to a 47.83% rise in root dry mass compared to the control with the cadmium concentration in the upper part reaching 125.21 mg/kg [6]. As a result, utilizing *Solanum nigrum* in the purification of cadmium-contaminated soil is expected to boost cadmium absorption and enrichment by improving root growth and biomass, thus enhancing the efficiency of soil cadmium cleanup.

Most investigations on plant-microorganism remediation focus on introducing cadmium into uncontaminated soils in potted plants in controlled environments, which do not accurately reflect the origins of cadmium pollution in soils caused by industrial activities and mimic the natural conditions in which plants typically thrive outdoors. As a result, the findings from these previous studies are not easily applicable to real-world scenarios. Additionally, microorganisms that are resistant to cadmium are mostly found in heavily contaminated areas rather than in the root soil of non-contaminated plants. There is a dearth of research on using cadmium-resistant microorganisms from diverse sources to aid in phytoremediation of cadmium-polluted soil. This study utilized cadmium-tolerant bacterial strains isolated from cadmium-contaminated soil and the rhizosphere soil of *Solanaceae* plants, as well as standard bacterial strains, and inoculated them into the rhizosphere soil of *Solanaceae* plants grown in open-air pots. The aim was to analyze the effects of cadmium-tolerant bacterial strains from different sources on the remediation of cadmium-contaminated soil by *Solanaceae* plants, thereby providing technical support for the treatment of cadmium-contaminated soil. This research would benefit the development of new combined technologies for heavy metal pollution control and the creation of new products.

## Materials and methods

### Soil sample collection and preparation

Cadmium-contaminated soil was collected from cultivated land in Wuhan, Hubei, China according to GPS coordinates. Soil samples were taken at a depth of 0 to 20 cm using the snake method and a stainless-steel small soil shovel. Ten samples were collected and placed into PVC plastic bags with a total of 320 kg. The samples were brought back to the laboratory, air-dried, ground, mixed thoroughly, sieved through a 2 mm nylon sieve, and prepared as comprehensive test samples. *Solanum nigrum* seeds (Hejian Fuyichun Seed Sales Co., Ltd., Cangzhou, Heibei, China) were planted and grown to obtain the *Solanum nigrum* rhizosphere soil and isolate the bacterial strains, while soil contaminated with cadmium was also used for strain isolation. The fresh collected soil samples were placed in kraft paper bags, stored in container with ice packs, and transported back to the laboratory where they were kept at 4℃. The quality control strain of *Pseudomonas fluorescens* was obtained from Zhilizhongte Biotechnology Co., Ltd., Wuhan, Hubei, China) [14]. A total of 10.0 g of fresh cadmium-contaminated soil and solanum nigrum rhizosphere soil was weighed, respectively. The soil samples were placed in 250 mL conical bottles containing 90 mL of sterile water and glass beads (National Pharmaceutical Group Chemical Reagent, Wuhan, Hubei, China) and then cultured in an oscillation of 150 rpm for 10 minutes. After standing for 30 minutes, 1 mL of the soil diluent was absorbed by an aseptic straw and placed in a 9 mL sterile water test tube to create a $10^{-1}$ soil diluent followed by series dilutions to prepare soil dilution with the concentration gradients of $10^{-2}$, $10^{-3}$, and $10^{-4}$.

**Bacterial purification and identification**

The supernatant from soil dilutions was plated onto 30 mL of beef extract peptone medium (National Pharmaceutical Group Chemical Reagent, Wuhan, Hubei, China) and incubated at 28℃ for 72 hours. Single colonies exhibiting diverse morphologies were identified and further purified to isolate single colonies displaying serpentine patterns. Screening of cadmium-resistant strains was performed using beef extract peptone medium containing cadmium ($CdCl_2·2.5H_2O$) (National Pharmaceutical Group Chemical Reagent, Wuhan, Hubei, China) at concentrations of 50 mg/L and 100 mg/L [15]. Purified single colonies and control *Pseudomonas fluorescens* were cultured in a serpentine line at 28℃ for 48 hours with 3 replicates per cadmium concentration. The bacteria capable of growing colonies were identified as cadmium-resistant strains. The siderophore-producing strains were screened by inoculating single colonies of cadmium-resistant strains in 2.645 mg/L chrome blue S (CAS) detection medium (Haibo Biotechnology Co., Ltd., Qingdao, Shandong, China) with platinum silk serpentine lines and cultured in a constant temperature incubator at 28℃ for 72 hours [15]. The strains exhibiting orange halos were identified as siderophore-producing strains [16]. The isolated cadmium-resistant and siderophore-producing bacteria were utilized as specific microbial agents for enhancing the remediation of cadmium-contaminated soil by *Solanum* and identified through observation of colony and cell morphology, as well as main physiological and biochemical tests [17], in addition to 16S rRNA gene sequence homology analysis. The cadmium-resistant iron-producing bacteria strains were attached to freeze-dried magnetic beads and submitted to Shanghai SBIO (Shanghai, China) for polymerase chain reaction (PCR) amplification of 16S rRNA gene with the primers of 27F (AGA GTT TGA TCM TGG CTC AG) and 1492R (GGT TAC TGT TAC GAC TT). The PCR amplification reaction included 5.0 μL of 2× PCR Premix, 0.5 μL of each 10 μmol/L primer, and 4.0 μL of bacterial template. The PCR amplification program was 95℃ for 2 min followed by 40 cycles of 94℃ for 5 s, 60℃ for 30 s, and 60℃ final extensions for 2 min. The PCR products were then sequenced, and the resulting sequences were compared with the GenBank database to identify homologies [18].

**Analysis of organic acids**

The analysis of organic acids secreted by cadmium-resistant strains was conducted in accordance with the "National Food Safety Standard for Determination of Organic Acids in Food" (GB 5009.157-2016). The seeds of

nightshade were initially germinated in seedling cups within a greenhouse environment. When the seedlings grew to 7 - 8 leaves, the stable seedlings were selected and transplanted into plastic pots that were 11 cm high and 18 cm in diameter with 1,000 g cadmium-contaminated soil. Each pot contained one plant and was positioned on the rooftop of a building, away from other potential sources of pollution. The plants were cultivated in open pots without base fertilizer or canopy. Deionized water was sporadically provided based on the soil's moisture levels over a two-week period. Once the plants established themselves, 10 mL of bacterial liquid with an optical density at 600 nm ($OD_{600}$) close to 0.5 was introduced to the plant roots and inoculated weekly. Seven different treatments were performed including a control group without bacterial inoculation (CK), inoculation of cadmium-resistant bacteria in cadmium-contaminated soil (T1), inoculation of cadmium-resistant bacteria in solanum nigrum rhizosphere soil (T2), inoculation of cadmium-resistant bacteria from *Pseudomonas fluorescens* (T3), inoculation of cadmium-resistant bacteria in cadmium-contaminated soil (T1 + T3), inoculation of cadmium-resistant bacteria in *Solanum nigrum* rhizosphere soil (T2 + T3), and inoculation of cadmium-resistant bacteria from cadmium-contaminated soil, *Solanum nigrum* rhizosphere soil, and *Pseudomonas fluorescens* (T1 + T2 + T3). Three replicates were performed for each treatment. Plastic trays were placed under the pots to prevent the leaching and leakage of heavy metal cadmium, and the leaking liquid was poured back into the pots. The plants were harvested after 120 days of growth. After gently pulling up the plants, the roots were shaken, and rhizosphere soil was collected in cloth bags or self-sealing bags and taken back to the laboratory to air dry naturally. The samples were ground and screened with particles less than 2 mm and mixed well. The particles were further reduced to less than 0.15 mm. The root soil of *Solanum nigrum* was cleaned, and the root system was carefully washed to obtain a complete plant before the plant height was measured, numbered, and photographed. The washed plant was then placed into a constant temperature drying oven at 105℃ for 5 minutes followed by drying at 70℃ for 48 hours. The plant dry mass of both aboveground and underground parts was weighed and further ground and sieved with the particle less than 0.85 mm and further reduced to less than 0.43 mm for analyzed samples. The cadmium in soil samples was determined using the method of DZ/T 0279.5-2016 “Regional Geochemical Sample Analysis Method Part 5: Determination of Cadmium Amount by Inductively Coupled Plasma Mass Spectrometry”. The available cadmium content was extracted using the diethylenetriamine pentaacetic acid extraction method. The total amount of cadmium in plant samples was determined using the method of "National Food Safety Standard Determination of Cadmium in Food" (GB 5009.15-2014).

**Data processing and analysis**

The enrichment coefficient was calculated to reflect Nightshade's ability to absorb heavy metals from the soil [19]. The transport coefficient was calculated to characterize its ability to transport heavy metals from underground to above ground [20]. The remediation efficiency (cadmium removal rate) was calculated to characterize the ability of heavy metals in the soil to be activated, absorbed, and transformed [21, 22]. WPS 2019 software (Beijing Kingsoft Office Software Co., Ltd., Beijing, China) was used for data processing, while SPSS 26.0 (IBM, Armonk, New York, USA) was employed for the significance analysis.

## Results and discussion

**Soil sample components**

The soil sample consisted of 68.4 g/kg organic matter content, 2.95 g/kg total nitrogen content, 2.17 g/kg total phosphorus content, and 10.83% total iron content by mass. The pH of the soil was determined to be 6.12 with a soil water content of 45.35%. The total cadmium concentration in the soil was 13.87 mg/kg with the available cadmium concentration at 3.27 mg/kg. The total

**Table 1.** Physiological and biochemical properties of identified cadmium-resistant bacterial strains.

| Index | Strain number | | |
|---|---|---|---|
| | **2021Z-4** | **2021Z-8** | **2021Z-PA1** |
| Cell shape | Rod | Rod | Rod |
| Gram stain | – | + | – |
| Contact enzyme activity | + | + | + |
| Oxidative fermentation of glucose | – | – | + |
| Oxidase activity | – | – | + |
| Species | *Bacillus velezensis* | *Pseudoclavibacter helvolus* | *Pseudomonas fluorescens* |
| Homology | 99.99% | 99.99% | 99.99% |
| Accession number | CP053764.1 | HM584267.1 | CP041771.1 |

cadmium concentration exceeded the risk control value specified in the "Soil Environmental Quality Agricultural Land Soil Pollution Risk Management (Trial)" (GB 15618-2018) (China Ministry of Ecology and Environment, Beijing, China) by nearly seven times.

**Screening and identification of cadmium-resistant strains**

The results demonstrated that 7 strains of bacteria were obtained from cadmium-contaminated soil, while 15 strains were obtained from nightshade rhizosphere soil. After the tolerance test with 50 mg/L cadmium, 7 strains including 2 from cadmium-contaminated soil and 5 from *Solanum solanum* rhizosphere soil were identified. Further, five strains with 2 from cadmium-contaminated soil and 3 from *Solanum solanum* rhizosphere soil demonstrated growth in 100 mg/L cadmium. Seven siderophore-producing strains were identified in CAS culture medium including 4 strains (2021Z-4, 2021Z-6, 2021Z-8, 2021Z-1-2) in 100 mg/L cadmium and 3 strains (2021Z-5, 2021Z-7, 2021Z-11) in 50 mg/L cadmium with two strains of 2021Z-4 and 2021Z-8 being selected. Moreover, two strains of *Pseudomonas fluorescens* (2021Z-PA1 and 2021Z-PA2) resistant to cadmium-producing siderophores were identified, which were tolerant to 50 mg/L and 100 mg/L cadmium contamination, respectively. Strains 2021Z-4, 2021Z-8, and 2021Z-PA1 were chosen for further physiological and biochemical studies, as well as bacterial identification with the main physiological and biochemical characteristics of the strains shown in Table 1.

**Effect of cadmium-resistant strains on the growth of *Solanum nigrum***

The results found that T1, T2, T2 + T3, and T1 + T2 + T3 demonstrated the accumulation enhancement of the dry matter in the underground portion of nightshade, suggesting that inoculation treatments could stimulate the development of the nightshade root system. In comparison to the control group (CK), the dry matter content of the above-ground and underground parts significantly increased by 31.18% and 75.61%, respectively, in the T2 + T3 treatment group, indicating the potential of inoculation treatment to enhance the growth of nightshade in cadmium-contaminated soil. However, T3 treatment appeared to impede the growth of nightshade (Figure 1).

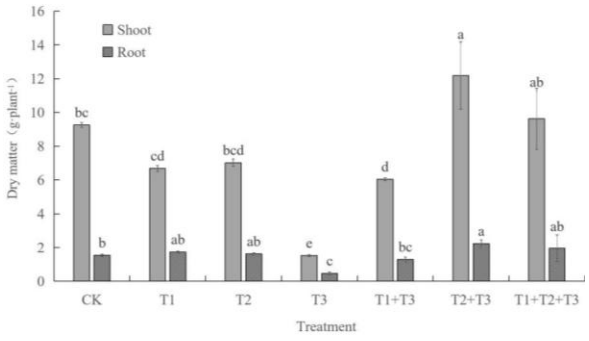


**Figure 1.** The dry weight of the aerial and underground parts of *Solanum nigrum* measured after inoculation with various cadmium-resistant strains. Different letters of the same index indicated significant differences between treatments ($P < 0.05$).

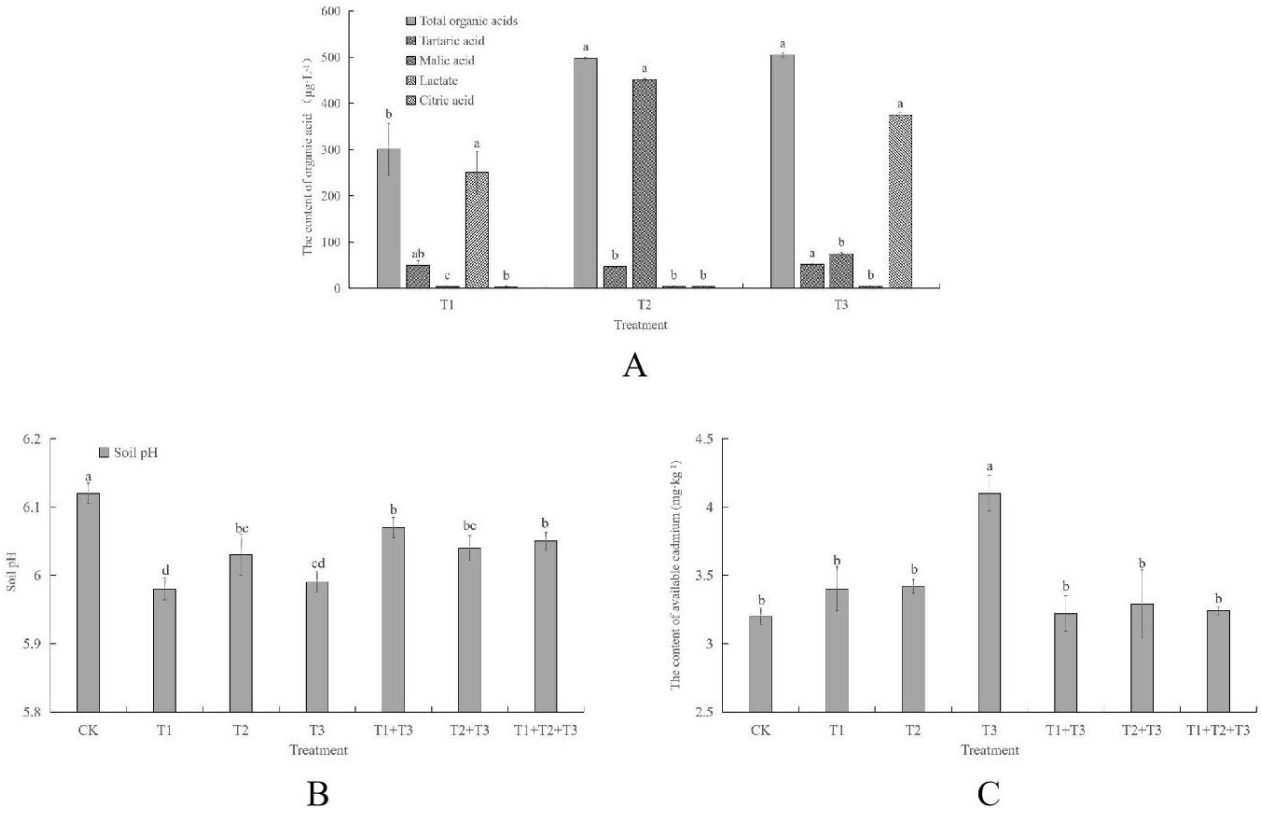


**Figure 2.** The contents of organic acids secreted by three single strains (**A**). Soil pH under inoculation with different cadmium-resistant strains (**B**). Available cadmium content in soil under different cadmium-resistant strains inoculated (**C**).

Previous research suggested that *Pseudomonas* secreted siderophores, which could enhance the absorption of essential nutrient elements such as iron and phosphorus in sweet sorghum, thereby facilitating its growth [11]. *Pseudomonas fluorescens* secreted the plant hormone indoleacetic acid and produced siderophores to enhance the growth of Sedum southeast [14]. Further, a significant amount of organic acids was secreted to facilitate the solubilization of nutrients. The light density of T3 + T2 strains was observed to be higher than T1, indicating a higher abundance of *P. pallidum* and *P. fluorescens* in the rhizosphere soil of nightshade. These strains exhibited stronger siderophore production capabilities, promoting the growth of nightshade. The T3 strain identified as *Pseudomonas fluorescens* demonstrated a robust siderophore secretion ability and strong organic acid secretion capabilities, which led to increased dissolution of heavy metals in the soil, enabling *Solanum nigrum* to absorb significant amounts of heavy metals, thereby impeding its own growth.

**Effects of organic acids secreted by cadmium-resistant strains on soil pH and available cadmium content**

The results showed that T1 strain mainly secreted lactic acid, while T2 strain mainly secreted malic acid, and T3 strain mainly secreted citric acid (Figure 2A). The total amount of organic acids secreted by T3 strain was significantly higher than that of T1 strain. Compared with CK, the soil pH of all inoculated treatments decreased significantly with T1 and T3 decreased by 0.14 and 0.13, respectively (Figure 2B). After inoculation, the available cadmium content in soil increased in different degrees compared with CK. The available cadmium content in T3 soil increased significantly by 26.76% (Figure 2C). The acid-producing ability of the strain could reflect its ability to activate heavy metals.

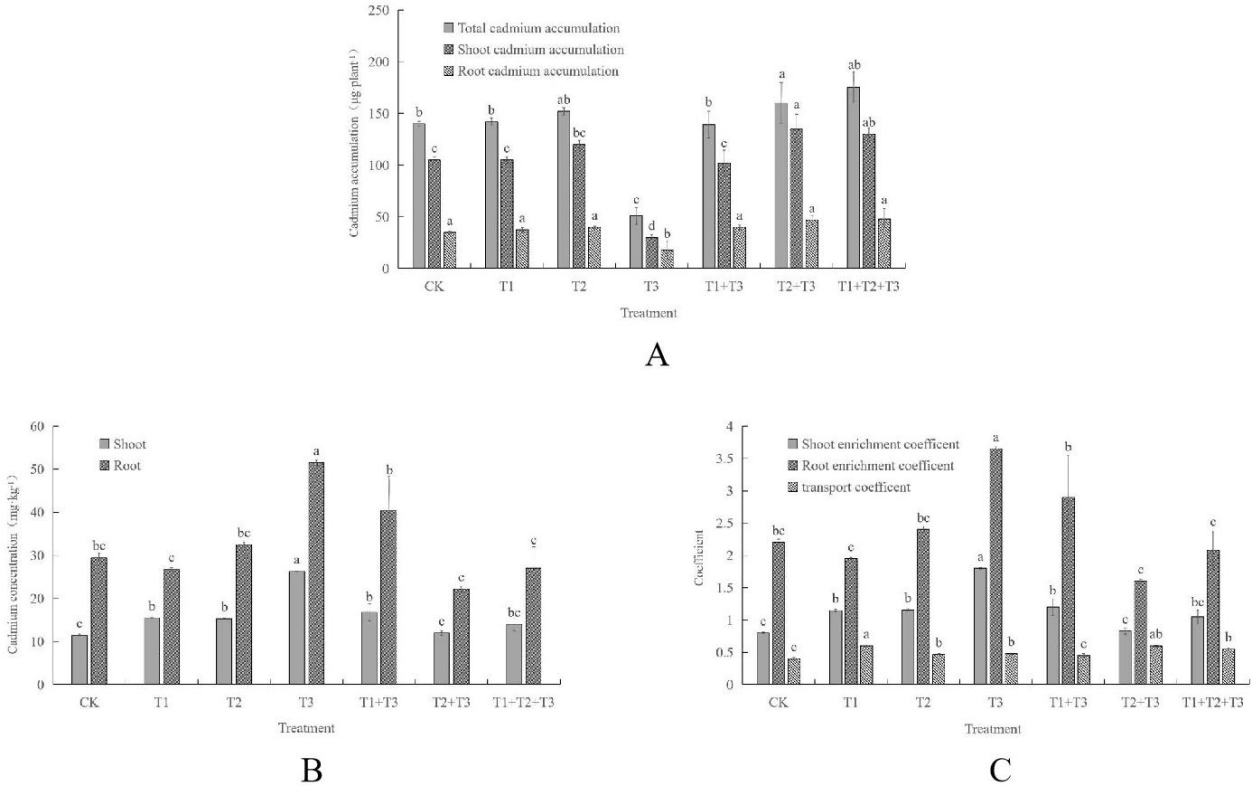


**Figure 3.** Cadmium accumulation in aerial and underground parts of *Solanum nigrum* inoculated with different cadmium-resistant strains (**A**). Cadmium concentration in shoot and root of *Solanum nigrum* inoculated with different cadmium-resistant strains (**B**). Enrichment coefficient and transport factor of *Solanum nigrum* inoculated with different cadmium-resistant strains (**C**).

Deng *et al*. reported that *Bacillus megaterium* metabolized organic acids during plant growth, which could activate soil cadmium to a certain extent, thereby increasing the content of available cadmium in soil [23]. Jiang *et al*. showed that *Pseudomonas* made the content of available cadmium in soil 16.63 times higher than that of the control group with the rate of activated cadmium in soil as 48.65% due to the production of a large number of acidic substances in the metabolic process of the strain, which activated the heavy metals originally in the precipitated state to become soluble metal ions [24]. The total amount of organic acids secreted by T3 strain was higher than that of T1, while the soil pH of T3 strain was significantly lower than that of CK strain, and the content of available cadmium in soil was significantly higher than that of other treatments with the total amount of activated cadmium in soil as 1,480.00 ± 400.37 μg/pot, which was higher than that of previously reported results, indicating that T3 strain had obvious advantages in secreting organic acids and activated cadmium.

**Effect of cadmium-resistant strains on cadmium accumulation in *Solanum nigrum***

The accumulation of cadmium is the product of biomass and cadmium content. Compared with CK, the total cadmium accumulation of T2, T2 + T3, and T1 + T2 + T3 solanum increased significantly by 7.60%, 30.39%, and 25.40%, respectively, with the total cadmium accumulation of T2 + T3 solanum as the highest one reaching 184.11 μg/plant (Figure 3A). The result was lower than previous research result that available cadmium was added to clean soil and carried out pot cultivation in greenhouse, which was not affected by leaching, and the plants had better absorption capacity for the original available cadmium [11]. When T2 + T3 solanum was leached, the activated cadmium

was lost with leaching, and the available cadmium absorbed by solanum was reduced, resulting in the total accumulation of cadmium in solanum being lower. Although T3 strain had a strong ability to secrete organic acids, the increase of available cadmium content in soil hindered the growth of nightshade and reduced the biomass of nightshade, so the total accumulation of cadmium in nightshade is the lowest. Both T2 + T3 and T1 + T2 + T3 significantly increased cadmium accumulation in the aerial part of *Solanum nigrum* by 31.64% and 25.22%, respectively, compared with CK. The accumulation of cadmium in underground part increased to some extent, but there was no significant difference compared with CK. The results showed that most of the cadmium absorbed by nightshade was accumulated in the aboveground parts, and T2 + T3 had a more significant promoting effect on the accumulation of cadmium in the aboveground parts of nightshade. Compared to the control group (CK), the cadmium content in both the aboveground and underground parts of T3 *Solanum nigrum* showed a significant increase of 129.29% and 73.19%, respectively (Figure 3B). Moreover, the enrichment coefficients of the above-ground and underground parts of T3 *Solanum nigrum* also exhibited significant increments of 128.40% and 72.90%, respectively (Figure 3C). Zhang *et al*. validated that *Pseudomonas* had the capability to activate cadmium in soil, leading to a significant rise in cadmium content in the aerial parts and roots of plants [15]. Similarly, Jiang *et al*. indicated that *Pseudomonas* could effectively activate precipitated cadmium in soil, thereby increasing the content of water-soluble cadmium in the soil [24]. In the current study, the PA strain was identified as *Pseudomonas fluorescens*, which secreted abundant organic acids that exhibited a potent activation effect on soil cadmium. Consequently, this strain enhanced the absorption of cadmium by *Solanum nigrum*, subsequently elevating the cadmium content and enrichment coefficient of the plant. Except for T1 + T3, the transport coefficients of the other inoculation treatments for *Solanum nigrum* were significantly higher than those of the control group (CK). The T1, T2, T3, T2 + T3, and T1 + T2 + T3 treatments resulted in increases of 52.63%, 26.32%, 31.58%, 39.58%, and 31.58%, respectively. These results suggested that inoculating cadmium-resistant bacteria into cadmium-contaminated soil could enhance the transport of cadmium from the underground parts of *Solanum nigrum* to the aboveground parts. The T1 strain, being indigenous to cadmium-contaminated soil, played a crucial role in this process. Additionally, the T2 strain identified as *Pseudomonas fluorescens* exhibited a potent activation effect on soil cadmium. The combination of T1 + T3 could effectively activate a substantial amount of cadmium in the soil, leading to enhanced absorption by the root system of *Solanum nigrum*. This increased absorption of activated cadmium triggered a response within the plant, resulting in hindered growth and reduced cadmium absorption in the aerial parts of *Solanum nigrum*, thus lowering the transport coefficient.

**Effects of cadmium-resistant strains on soil cadmium content and remediation efficiency**

The cadmium levels in all treated soils were lower than those prior to remediation (138.7 mg/kg). Among them, the cadmium content in soil treated with T2 + T3 decreased by 41.3% compared to the control group, and the remediation efficiency increased significantly by 67.93% compared to the control (Figure 4). It was evident that T2 + T3 enhanced the remediation of cadmium-contaminated soil by *Solanum nigrum*, exhibiting the highest biomass and total cadmium accumulation of *Solanum nigrum*, thus achieving the best remediation efficiency for cadmium-contaminated soil. This finding aligned with the conclusion of Ma *et al*., which endogenous bacteria could help host plants increase their metal tolerance, biomass, and heavy metal absorption, ultimately enhancing the concentration of heavy metals in plants and improving phytoremediation efficiency [25]. The T2 strain, an endogenous bacterium found in *Solanum nigrum* root soil, and the T3 strain, an exogenous bacterium, exhibited strong abilities in producing siderophore and organic acid, which

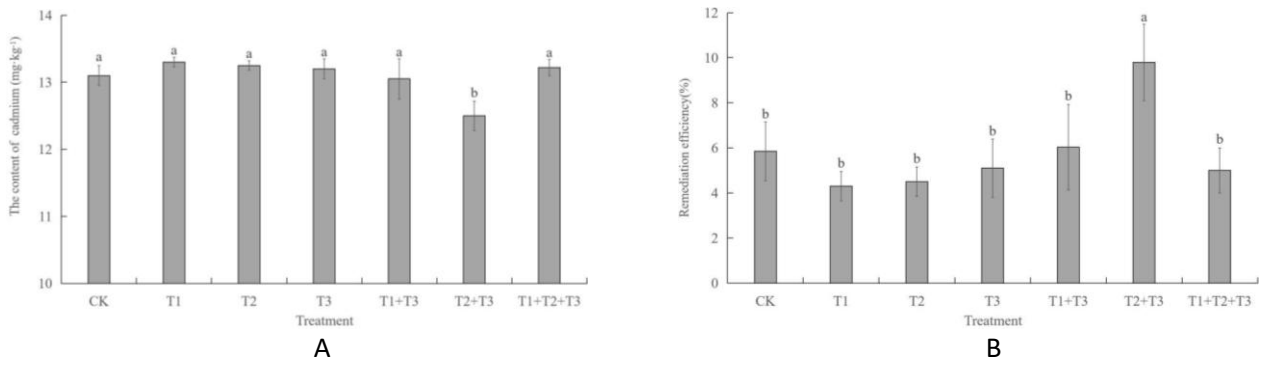


**Figure 4.** Soil cadmium content (**A**) and remediation efficiency (**B**) with different cadmium-resistant strains.

collectively contributed to the improved remediation efficiency of *Solanum nigrum* on cadmium-contaminated soil when used in combination as T2 + T3. The amount of leached cadmium was calculated by subtracting the total amount of cadmium in the soil before remediation from the sum of the amount of cadmium accumulated in the remediated nightshade and the total amount of cadmium in the remediated soil. Despite placing a plastic tray beneath the basin, cadmium leaching and leakage were expected in seasons characterized by heavy rainfall. A Pearson correlation coefficient of 0.98 between soil remediation efficiency and leached cadmium was obtained, which suggested a significant positive relationship between soil remediation efficiency and leached cadmium. Specifically, 13.84% of the cadmium removed from the T2 + T3 soil was absorbed and accumulated by *Solanum nigrum*, while the remaining 86.27% was lost due to leaching. This result highlighted the considerable impact of leaching on soil remediation. If this method is implemented in arid land or fields, there may be potential hazards to deep soil and groundwater due to the high rate of cadmium leaching. Therefore, further research focusing on the disposal of cadmium leaching risks is imperative. The auxiliary materials that can activate cadmium in the soil, remain stable in the soil, and be absorbed by plants should be explored.

**Relationship between soil cadmium availability and soil remediation efficiency**

The presence of heavy metals in soil significantly impacts the uptake of heavy metals by plants, thus influencing the effectiveness of phytoremediation [26]. In comparison to the control group (CK), the T3 strain secreted large amounts of organic acids, leading to a significant decrease in soil pH post-treatment. Additionally, the levels of available cadmium in the soil, as well as the cadmium content and enrichment coefficient in *Solanum nigrum*, were all high in the T3-treated soil. However, the soil remediation efficiency was low in the T3 treatment group. This discrepancy could be attributed to the similar amounts of cadmium leached in both the T3 and CK treatments with the high secretion of organic acids from the T3 strain promoting the activation of soil cadmium. The increase in soil cadmium availability might inhibit the growth of *Solanum nigrum* to some extent. Consequently, the reduced biomass of *Solanum nigrum* led to lower total cadmium accumulation, resulting in lower soil remediation efficiency compared to the CK treatment. The soil pH was lower in T2 + T3 treatment than in CK. The soil available cadmium content and cadmium content in the aboveground part of *Solanum nigrum* did not show significant increases, while the cadmium content in the underground part was lower. However, the soil remediation efficiency was significantly improved in the T2 + T3 treatment, which could be attributed to several factors including that T2 strains acted as

endogenous bacteria, enhancing heavy metal tolerance and biomass of host plants, while T3 strain exhibited strong organic acid secretion capabilities, leading to increased cadmium availability in the soil, and the increase in leached cadmium reduced the overall cadmium content in the soil, contributing to a substantial enhancement in soil remediation efficiency.

## Conclusion

This study screened cadmium-resistant bacterial strains from cadmium-contaminated soil and *Solanum nigrum* rhizosphere soil and inoculated standard strains into *Solanum nigrum* rhizosphere soil to investigate the enhancing effect of cadmium-resistant strains from different sources on the remediation of cadmium-contaminated soil by *Solanum nigrum*. A cadmium-resistant microorganism was isolated from cadmium-contaminated soil and identified as *Bacillus velez*, while another cadmium-resistant microorganism was isolated from the rhizosphere soil of *Solanum nigrum* and identified as *Pseudoscorynebacterium flavus*. Further screening resulted in the isolation of *Pseudomonas fluorescens* strains with similar cadmium-resistant siderophore activity. The efficiency of soil remediation was determined by the accumulation of cadmium and the leached cadmium in *Solanum nigrum*. Soil cadmium availability refers to the ability of microorganisms to secrete organic acids to activate cadmium including cadmium accumulation and leached cadmium in *Solanum nigrum*, as well as excess activated cadmium in the soil. Therefore, by minimizing cadmium leaching, increasing the availability of cadmium in the soil, and enhancing the biomass of nightshade, cadmium accumulation in nightshade and its efficiency in remediating cadmium-contaminated soil could be improved.

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