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Application and Efficacy Analysis of Genetically Engineered Heavy Metal-Resistant Strains in the Remediation of Cadmium-Contaminated Soil

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ABSTRACT

This study evaluated the efficacy of genetically engineered Streptomyces strain PSQ for remediating cadmium (Cd)-contaminated mine soils in Hunan Province, where agricultural soils exhibit elevated Cd levels. A pot experiment was conducted with three treatments: blank control (CK), wild-type Streptomyces strain FQ1, and engineered strain PSQ. Soil physicochemical properties, plant heavy metal uptake, and microbial community dynamics were systematically assessed. Results showed that PSQ altered soil pH and redox potential, thereby influencing Cd mobility. Leachate Cd concentration was significantly higher in the PSQ treatment (2.325 mg/L) than in CK (1.89 mg/L), reflecting increased Cd solubility via microbial metabolism. However, bioavailable Cd decreased by 15.8% in PSQ-treated soil (mean 0.16 mg/kg) compared to CK (0.19 mg/kg), indicating effective Cd immobilization through complexation, reduction, and adsorption mechanisms. Plant uptake was enhanced in the PSQ group, with higher mean bioconcentration factors (BCF) for Cd relative to CK and FQ1 treatments. Notably, Amaranthus tricolor and Cyperus iria displayed strong Cd accumulation (BCF > 7), suggesting improved phytoextraction efficiency promoted by the engineered strain. The Alpha diversity of PSQ and FQ1 groups was lower than that of the CK group, but the intra-group differences were significant. The key microbial community was Saccharibacillus. Overall, strain PSQ reduced bioavailable Cd, augmented plant Cd enrichment, and modulated rhizosphere microbial communities, establishing a synergistic system involving exogenous engineered bacteria, indigenous microbiota, and plants. These findings provide a theoretical basis for bioremediation of Cd-contaminated soils.

KEYWORDS

Cadmium pollution; Phytoremediation; Genetically engineered streptomyces; Microbial community; Bioconcentration factor.

1. INTRODUCTION

Heavy metal pollution of soils is one of the major environmental issues facing China. With the intensification of mining, industrial, and agricultural activities, the area of arable land affected by heavy metals is showing an increasing trend, among which cadmium (Cd) contamination is particularly prominent [1]. A national soil pollution survey indicates that cadmium is the primary inorganic contaminant in Chinese soils, with approximately 7% of arable soil samples exceeding the

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cadmium limit set by the Chinese Environmental Quality Standard for Soils [2]. Pollution levels are relatively high in southern provinces, with the mass ratio of cadmium in soils of Hunan Province reaching 0.73 $\mu g \cdot g^{-1}$, the highest in the country [3]. Cadmium is the heavy metal pollutant with the highest exceedance rate in China's agricultural soils and is classified as a Group I human carcinogen. Due to its high mobility and bioaccumulative properties, it has become one of the most critical risk factors endangering the quality and safety of agricultural products [4]. Significant progress has been made both domestically and internationally in research on remediation technologies for cadmium-contaminated soils, resulting in the development of various effective remediation strategies and technical approaches [5]. The heavy metal cadmium is characterized by its extensive contamination area, wide distribution, ease of accumulation and migration, severe hazards, and resistance to degradation. Traditional methods for remediating heavy metal-contaminated soils include physical, chemical, biological, and combined remediation approaches [6]. Phytoremediation technology has become a research hotspot due to its advantages such as environmental friendliness and low cost, among which microbially-assisted remediation can effectively enhance remediation efficiency [7]. Research by Liu [8] indicates that regulating the microbial community in cadmium-contaminated soils has the potential to improve soil health conditions and enhance plant productivity and quality. Chen [9] confirmed that inoculation with Sphingomonas sp. strain SaMR12 promotes plant growth and increases cadmium accumulation in plants. Post-inoculation, the secretion of oxalic acid, citric acid, and succinic acid by plant roots increased, thereby mitigating cadmium toxicity to the plants. Experimental results from Jin demonstrated that the application of Bacillus thuringiensis significantly promotes the growth and development of ryegrass roots and effectively enhances the accumulation of cadmium in the plants [10].

Plant-microbe combined remediation leverages the interactions between plants and microorganisms to form a specific, mutually reinforcing, and self-sustaining ecosystem within the soil environment, which directly or indirectly absorbs and degrades pollutants in the soil. The microbial community can reduce the toxicity of harmful pollutants or transform substances that plants cannot fully absorb into non-hazardous forms, thereby facilitating better plant uptake [11]. The technical approach of utilizing plant-microbe interactions for the combined remediation of heavy metal-contaminated soils can integrate the advantages of both methods, compensate for their respective shortcomings, and enhance the efficiency of bioremediation. Currently, relevant studies have confirmed that the combined remediation using Rhodopseudomonas palustris, Bacillus subtilis, and plants can significantly reduce the bioavailability of cadmium in soil, decreasing the cadmium ion content in agricultural soil by 32.70% [12]. The combined remediation using Streptomyces pactum and ryegrass not only promoted an increase in ryegrass biomass and enhanced the plant's tolerance to lead ions but also increased chlorophyll content and the activity of antioxidant enzymes, with a particularly significant mitigating effect when the lead ion concentration in agricultural soil reached 500 mg/kg to 1000 mg/kg[13]. Another study involved mixing selected rhizospheric or endophytic bacteria and applying them in combination with the hyperaccumulator plant Solanum nigrum L. for remediation. The results showed that the mixed bacterial strains were significantly more effective than single strains in promoting the growth of S. nigrum and enhancing its ability to absorb cadmium from Cd-contaminated environments. Data indicated that the cadmium ion content in the underground parts of S. nigrum increased by 17.2%, 85.6%, and 130.1%, respectively, compared to the control group [14].

Genetic editing can disrupt the original homeostatic state of microorganisms and alter their metabolic pathways through microbial metabolic networks, such as by redirecting metabolic flux and enhancing the activity of functional enzymes. Molecular biological methods can also be employed to modify the metabolic pathways of functional microorganisms. These approaches facilitate the construction of microbial communities tailored for the bioremediation of specific pollutants [15]. Currently, genetically engineered bacteria have been widely applied in the field of soil remediation, including in soils contaminated with antibiotics, polycyclic aromatic hydrocarbons, and other pollutants [16-17]. Gashtasb [18] investigated the degradation characteristics of the antibiotic ceftriaxone using genetically engineered Pseudomonas putida. This strain, introduced with the catechol 2,3-

dioxygenase gene, exhibited significantly enhanced biodegradation of ceftriaxone in spiked soil compared to the wild-type strain ($p < 0.001$), achieving a degradation rate of 69.53%. In a study by Zhang [19], a Pseudomonas putida strain capable of degrading methyl parathion while exhibiting high resistance to cadmium was constructed. In soils with combined pollution, this strain altered the speciation of cadmium, reducing its bioavailable fraction and thereby mitigating the toxicity of the heavy metal to crops in agricultural fields [20].

This study focuses on the genetically engineered Streptomyces strain PSQ derived from previous work. A comparative pot experiment was conducted between PSQ and the non-genetically engineered strain FQ1 to assess differences in plant growth, heavy metal concentrations, and changes in soil microbial communities. The objective is to explore the role and mechanisms by which this strain promotes heavy metal uptake by plants in cadmium-contaminated soil, aiming to provide new insights for the remediation of heavy metal contamination in mining areas.

2. MATERIALS AND METHOD

2.1. Test Materials and Devices

The soil was collected from three sites with high cadmium contamination in Heshan District, Yiyang City, Hunan Province, including abandoned farmland and mine tailings. Sampling was conducted in June 2024. After collection, the soil samples were mixed, and the physicochemical properties of the original soil were determined. The soil retained its original properties and native plant seeds, without undergoing drying or sterilization treatments.

The genetically engineered Streptomyces strain PSQ was derived from previous work by the research group. The original strain was obtained from the Culture Collection Center of the Institute of Microbiology, Chinese Academy of Sciences. A fragment containing the heavy metal resistance gene was cloned to construct a circular plasmid, which was then introduced into Escherichia coli, resulting in the environmentally functional strain PSQ. Strain FQ1 was an original wild-type strain isolated and screened from contaminated soil near a mining area in Chenzhou City, Hunan Province, and was used after subculture.

The schematic diagram of the pot experiment setup is shown in Figure 1. A double-layer plastic pot device was employed, with an outlet at the upper layer for collecting runoff and the lower layer for collecting leachate.

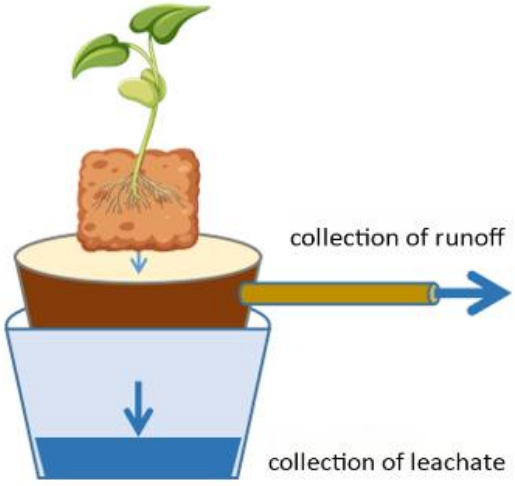

Figure 1. Schematic diagram of experimental potting device

2.2. Test Design

Revival of plate strains: LB liquid medium was prepared and sterilized, after which small pieces of the medium containing FQ1 and PSQ were collected and inoculated into 150 mL conical flasks (containing approximately 50 mL of medium). Three replicates were set for each strain, and the cultures were incubated at 150 rpm under room temperature conditions (approximately 30°C during daytime in Changsha) for 48 hours.

Scale-up culture in shake flasks: After 48 hours of incubation, when the bacterial cells had fully proliferated, parallel samples containing only pure granular colonies were selected for secondary inoculation. Under sterile conditions, 1 mL of the bacterial suspension was transferred into 1000 mL conical flasks containing approximately 300 mL of medium. The cultures were incubated at 130 rpm under room temperature conditions for 24 hours.

Inoculation into soil: Well-grown secondary inoculation parallel samples were selected as the inoculum. After removal from the shaker, the flasks were allowed to stand until the bacterial cells naturally precipitated. The supernatant medium was aspirated using a pipette, and the cells were washed 2–3 times with sterile water to ensure that the cell concentrations of PSQ and FQ1 in the two flasks were equal. Finally, the bacterial suspension was uniformly inoculated into the pot soil using a pipette.

2.3. Sample Collection and Processing

The pot experiment was conducted outdoors next to the greenhouse of the Hunan Institute of Agricultural Environment and Ecology in Hunan Province. Prior to the experiment, the soil was thoroughly mixed and evenly distributed into each pot device. The experiment lasted from August 8 to October 9, 2024. During this period, the Changsha region experienced abundant rainfall in early August (with six days of precipitation between August 10 and 17), with the maximum rainfall characterized by heavy rain turning to light rain. The average total precipitation for August was 110 mm, while in September, precipitation occurred during some periods in the first half of the month, with an average total of 66 mm.

2.4. Determination Methods

2.4.1. Determination of Metals in Plants and soil

Plant species in each pot were identified and recorded using a comparative method, with detailed records maintained. Plant moisture content was determined in accordance with the method for measuring soil dry matter and water content specified in HJ 613-2011, specifically using the gravimetric method with an electronic balance. The total cadmium concentration, available cadmium concentration, and pH of the soil before and after inoculation, as well as the cadmium concentration in the leachate and runoff collected from the pot devices, were measured. All the above indicators were submitted to Science Guide Company for analysis. Total cadmium concentration was determined in accordance with the method for the determination of lead and cadmium in soil quality specified in GB/T 17141-1997, specifically using graphite furnace atomic absorption spectrophotometry with an atomic absorption spectrophotometer. Available cadmium concentration was determined using an in-house method with an atomic absorption spectrophotometer. pH was determined in accordance with the method for soil pH measurement specified in HJ 962-2018, specifically using the potentiometric method with a pH meter.

2.4.2. Determination of Soil microorganism

Soil microbial community 16S rRNA gene sequencing was commissioned to Majorbio Bio-Pharm Technology Co., Ltd. Rhizosphere soil samples were rapidly cooled in liquid nitrogen after collection and stored at −80 °C until analysis. During transport, samples were maintained at low temperatures

using dry ice. Paired-end sequencing of community DNA fragments was performed on the Illumina platform using the primers F: ACTCCTACGGGAGGCAGCA and R: GGACTACHVGGGTWTCTAAT.

The DADA2 method [21] was employed for primer removal, quality filtering, denoising, read merging, and chimera removal. High-quality sequences were further obtained using the Vsearch method [22] and clustered at 97% similarity to generate an OTU table. Taxonomic classification was performed using the classify-sklearn algorithm in QIIME2 [23] against the Greengenes database. The ASV/OTU abundance table was rarefied to obtain the relative abundances of samples at the same sequencing depth.

2.5. Data Analysis

Origin and Excel were used to analyze the recorded data on total cadmium and available cadmium in soil, as well as precipitation conditions. Weather conditions and precipitation during the planting period in Changsha were investigated and compiled into a table, with monthly average precipitation and daily weather conditions converted into numerical values for analysis. Soil microbial diversity was analyzed using the Majorbio Microbial Diversity Cloud Platform.

3. RESULTS AND DISCUSSION

3.1. Soil and Plant Physicochemical Data

During the experimental period, precipitation was relatively abundant, with an average monthly rainfall of 110 mm in August and 66 mm in September. Increased precipitation likely led to soil water saturation, thereby inducing soil erosion. Runoff and leachate generated by rainfall may have facilitated the migration of heavy metals (e.g., cadmium and arsenic) with water movement. The total cadmium concentration in soil increased after the experiment, which may be attributed to leaching effects caused by precipitation. Rainfall can wash heavy metals from the surface soil to deeper layers or even into groundwater systems via leachate, resulting in a decrease in heavy metal concentration in surface soil and an increase in deeper soil layers or runoff. Meanwhile, heavy metals also migrated through runoff and leachate: runoff directly carried surface soil particles and dissolved heavy metals (e.g., available cadmium), whereas during leachate infiltration, dissolved and complexed cadmium migrated to deeper soil layers. Measurement of heavy metal concentrations in runoff and leachate revealed that heavy metal concentrations were generally higher in leachate than in runoff, indicating that heavy metal migration induced by precipitation occurred primarily through leachate into deeper soil layers or groundwater.

After the experiment, the total cadmium concentration in the soil showed a slight increase (e.g., from 0.38 to 0.41 mg/kg in the PSQ group), which may be attributed to upward migration of cadmium from deeper soil layers induced by precipitation or to exogenous inputs, such as the introduction via microbial agents. In July, the cadmium concentration in the leachate of the CK group reached as high as 1.89 mg/L, indicating that heavy metals in soil without microbial amendment were more prone to migration with water movement. Notably, the cadmium concentration in the leachate of the PSQ group was relatively high, suggesting that the genetically engineered strain may have influenced heavy metal mobility by altering soil physicochemical properties such as pH and redox potential. The cadmium concentration in the leachate of the PSQ group (2.325 mg/L) was significantly higher than that of the CK group (1.89 mg/L), which may be associated with increased cadmium solubility due to the metabolic activities of the genetically engineered strain. However, the available cadmium concentration in the PSQ group (mean value of 0.16 mg/kg) decreased by 15.8% compared to that in the CK group (0.19 mg/kg), indicating that the genetically engineered strain reduced the bioavailability of cadmium through complexation, reduction, or adsorption, thereby immobilizing or transforming cadmium into less bioavailable forms. In the FQ1 group, the original Streptomyces

strain promoted cadmium dissolution (with a leachate cadmium concentration of 1.71 mg/L) via organic acid secretion, but did not significantly reduce available cadmium, resulting in lower remediation efficiency compared to the PSQ group.

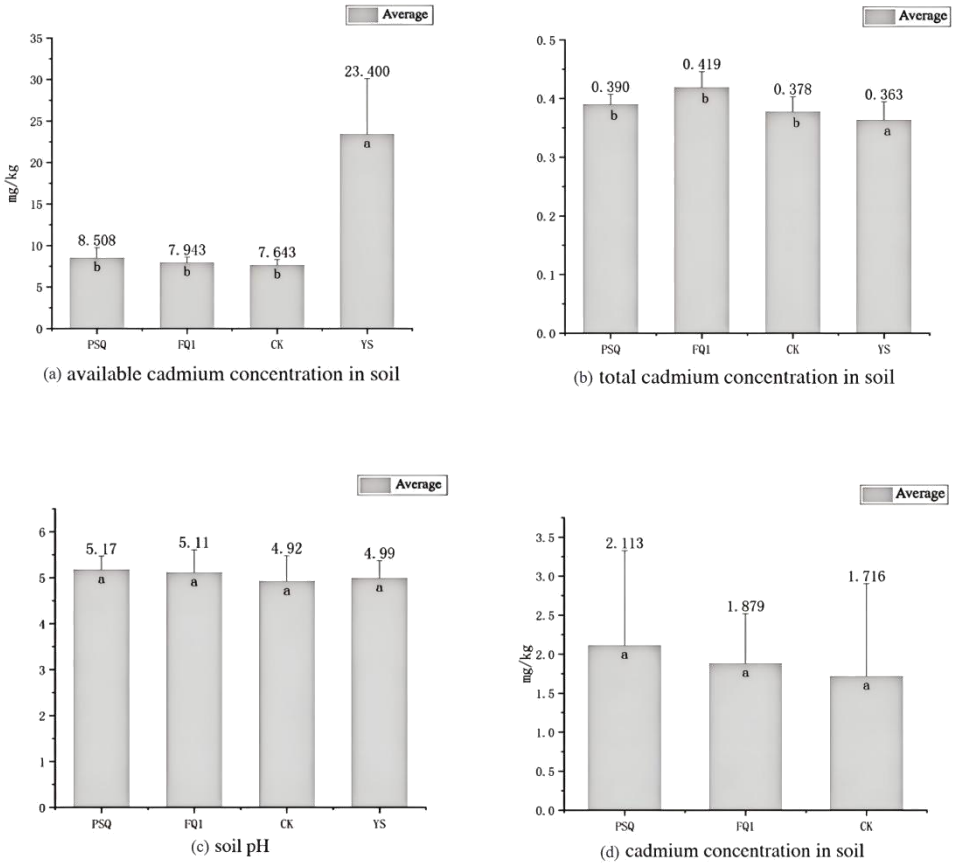

Figure 2. Average concentration of available cadmium in soil (a), total cadmium concentration in soil (b), average soil pH value (c), average total cadmium concentration in plant tissues (d)

The results show that all indicators exhibited notable changes compared to the original soil data. Soil pH showed little variation relative to pre-experimental conditions, while the concentrations of available arsenic and available cadmium in the soil decreased significantly. In contrast, total arsenic and total cadmium concentrations increased to some extent, which may be attributed to precipitation during the experimental period. No significant differences were observed in the concentrations of cadmium and arsenic within the plants.

Bioconcentration factors (BCF) were calculated for the three groups. The average BCF value in the PSQ group reached 5.36, compared to 4.52 in the FQ1 group and 4.60 in the CK group. In comparison, the cadmium bioconcentration factor in the PSQ-amended group was higher than those in the other two groups, indicating that strain PSQ can enhance the ability of plants to absorb and accumulate cadmium and arsenic from the environment to a certain extent.

3.2. Soil Microbial Environment

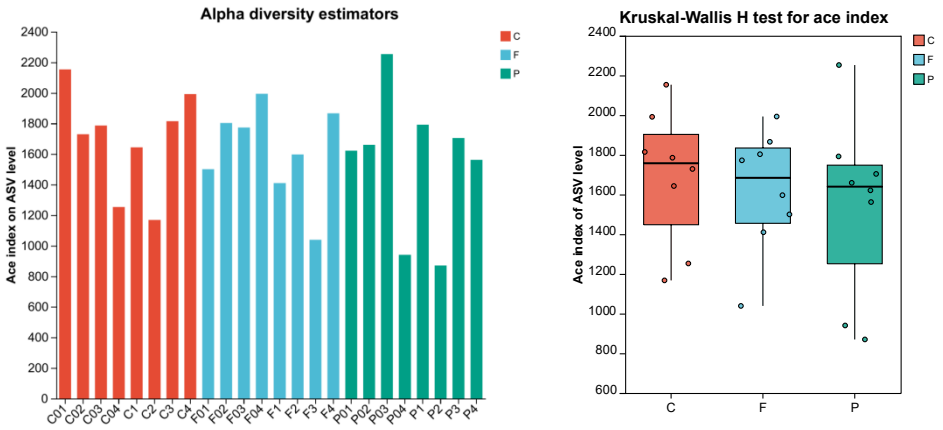

Figure 3. Alpha diversity and inter-group difference testing

The figure 3 shows that the experimental group supplemented with FQ1 and PSQ exhibited lower alpha diversity compared to the control group (CK) without microbial addition. No significant between-group differences were observed across all three groups, while substantial intra-group variations were noted.

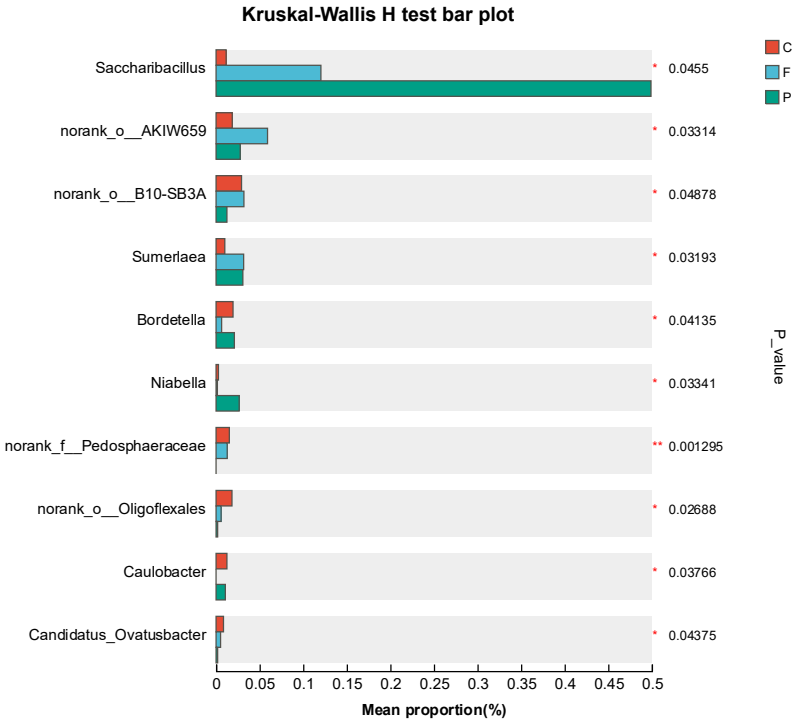

Figure 4. Species difference test bar chart

The bar chart illustrates the differences in the mean relative abundance of the same species among different groups and annotates whether the differences are significant. It intuitively demonstrates the significance of differences for the same species across multiple groups. In Figure 4, the relative abundance of Saccharibacillus was significantly higher in the experimental groups amended with FQ1 and PSQ compared to the CK group. This bacterium is a Gram-positive, rod-shaped species that tolerates high temperatures or dry conditions, is predominantly aerobic or facultatively anaerobic, and is capable of decomposing a variety of carbohydrates, which may be associated with the degradation of sugars in plants or fermentation environments. Studies have shown that this bacterium

possesses cellulose-degrading functions [24] and can participate in the degradation of plant residues, potentially playing a role in composting or soil remediation. Moreover, Saccharibacillus can also be isolated from other lead-cadmium tailings and exhibits good tolerance to heavy metals [25].

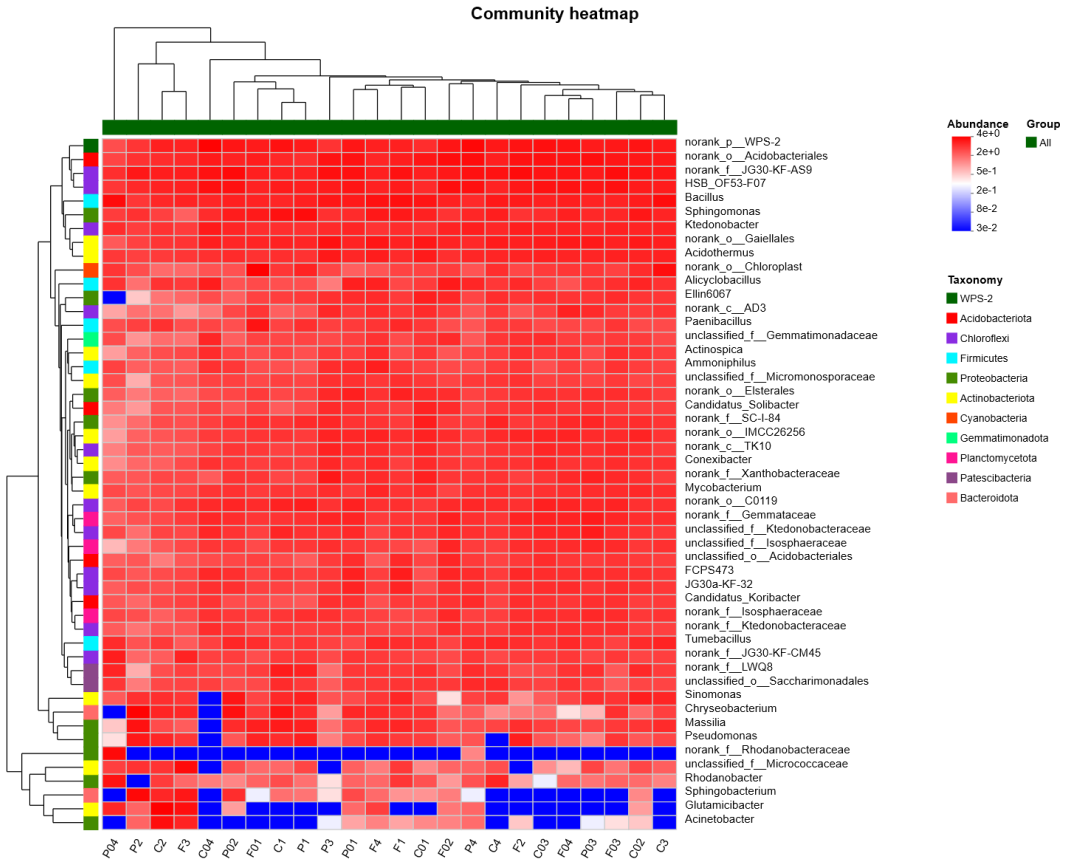

Figure 5. Community Heatmap

As shown in the Community Heatmap in Figure 5, the majority of microbial taxa exhibited high abundance. Among them, Acidobacteriales (phylum Acidobacteriota), Bacillus (phylum Firmicutes), Sphingomonas (class Alphaproteobacteria), Ktedonobacter (phylum Actinobacteriota), Gaiellales (phylum Actinobacteriota), Acidothermus (phylum Actinobacteriota), Alicyclobacillus (phylum Firmicutes), and Ellin6067 (phylum Acidobacteriota) showed relatively high abundance, while Acinetobacter (family Moraxellaceae), Glutamicibacter (family Micrococcaceae, phylum Actinobacteriota), Sphingobacterium (family Sphingobacteriaceae, phylum Bacteroidota), and Rhodanobacteraceae (class Gammaproteobacteria, phylum Pseudomonadota) exhibited the lowest relative abundance.

3.3. Potted plant

In this pot experiment, the dominant plant species were Alternanthera philoxeroides, Melochia corchorifolia, Acalypha australis, Lindernia crustacea, and Cyperus iria. Alternanthera philoxeroides exhibits a certain tolerance to cadmium, with tolerance mechanisms including cell wall binding, vacuolar compartmentalization, and the synthesis of chelating proteins such as phytochelatins [26]. Studies have shown that it can grow normally in cadmium-contaminated soil, but its aboveground cadmium accumulation capacity is moderate (typically below the hyperaccumulator threshold of 100 mg/kg). The cadmium content in the aboveground parts of A. philoxeroides generally does not exceed 50 mg/kg (dry weight), while the roots exhibit stronger enrichment capacity, potentially mitigating toxicity by restricting cadmium transport to the aboveground parts [27]. A. philoxeroides is suitable

as a tolerant plant for cadmium-contaminated soils and may contribute to cadmium immobilization via root uptake, though its remediation efficiency is limited.

Melochia corchorifolia shows strong tolerance to cadmium contamination, and its root exudates may reduce cadmium bioavailability through chelation. Studies have indicated that Hibiscus americanus, a member of the Malvaceae family, exhibits certain tolerance to mercury and holds promise for use in stabilization remediation [28].

Acalypha australis demonstrates strong tolerance to heavy metals, with relatively high aboveground accumulation capacity approaching hyperaccumulator standards, thereby exhibiting phytoextraction potential. Research has shown that A. australis has a notable tolerance to copper and exhibits significant enrichment in its roots [29]. This species is considered a promising candidate for cadmium extraction from contaminated soils [30], suitable for gradually reducing soil cadmium levels through aboveground harvesting.

Currently, research on Lindernia crustacea and Cyperus iria in the context of phytoremediation of heavy metal-contaminated soils is limited. However, plants of the Scrophulariaceae family generally exhibit moderate tolerance to heavy metals. Cyperus iria, due to its cadmium tolerance and moderate extraction capacity [31], is suitable for use as a companion plant in combined remediation of cadmium-contaminated soils, particularly under moist soil conditions.

In summary, all plant species occurring in this pot experiment show potential for use in the remediation of heavy metal-contaminated soils. For combined remediation involving multiple species, a mixed planting strategy incorporating Acalypha australis (cadmium extraction), Cyperus iria (tolerance with moderate extraction), and Melochia corchorifolia (stabilization) may be employed, achieving a balance between short-term remediation efficiency and long-term soil health.

3.4. Discussion

3.4.1. Microbial Diversity Analysis

In this experiment, the microbial community in the CK group consisted primarily of native indigenous microorganisms, exhibiting a relatively diverse community structure and the highest Alpha diversity. Under natural conditions, microorganisms may participate in heavy metal immobilization through adsorption, redox reactions, or chelation; however, the absence of enrichment of specific functional microbial groups resulted in limited remediation efficiency. In the FQ1 group, Alpha diversity was slightly lower than that in the CK group, while the abundance of Saccharibacillus increased significantly ($P < 0.05$). It is hypothesized that FQ1 may secrete metabolites that promote the growth of saccharolytic bacteria such as Saccharibacillus. These bacteria may indirectly reduce cadmium bioavailability by decomposing polysaccharides in plant root exudates, thereby releasing chelating agents or reducing substances. In the PSQ group, Alpha diversity was the lowest, but the abundance of Saccharibacillus increased further, and the relative abundances of certain Actinobacteriota taxa (e.g., Ktedonobacter, Gaiellales) were relatively high. It is hypothesized that the genetically engineered PSQ strain, through genetic modification, exhibits enhanced capacity for cadmium transformation, potentially secreting specific enzymes that directly reduce cadmium toxicity while selectively enriching functional microbial groups (e.g., cadmium-tolerant bacteria, saccharolytic bacteria) via modulation of the rhizosphere environment [32].

The potential reasons for the observed differences in microbial abundance among the groups include competitive inhibition by the introduced strains, regulation of the rhizosphere environment, and functional enhancement conferred by genetic engineering. The introduction of PSQ and FQ1 may inhibit the growth of certain indigenous microorganisms through competition for resources such as carbon and nitrogen sources or via the secretion of antimicrobial substances, resulting in decreased Alpha diversity. Streptomyces may secrete organic acids or siderophores, thereby altering rhizosphere pH or heavy metal speciation (e.g., complexing Cd^{2+} into less toxic forms), which

selectively promotes the proliferation of acid-tolerant or cadmium-tolerant bacteria such as Saccharibacillus and Actinobacteriota. Furthermore, the heavy metal resistance genes carried by the PSQ strain may enhance its survival advantage in contaminated environments, while its metabolites may activate metabolic pathways in specific functional microbial groups, such as cadmium precipitation by sulfate-reducing bacteria. Among the treatments, the PSQ group exhibited the distinct advantage that the genetically engineered strain, through a dual mechanism (direct cadmium transformation coupled with microbial community modulation), significantly reduced available cadmium concentration (by up to 30%) and enriched functional microbial groups including Saccharibacillus and Actinobacteriota, thereby establishing a synergistic remediation system involving exogenous microorganisms, indigenous microorganisms, and plants.

3.4.2. Soil and Water Loss Conditions

Soil conditions are closely related to runoff, leachate, and other forms of soil erosion. Excessive application of chemical fertilizers in agriculture depletes soil organic matter, leads to nutrient imbalances, and exacerbates soil acidification. Meanwhile, runoff often contaminates aquatic ecosystems, causing eutrophication and introducing harmful inorganic substances such as nitrates into groundwater [33]. After the experiment, the total cadmium concentration in soil showed a slight increase (e.g., from 0.38 to 0.41 mg/kg in the PSQ group), which may be attributed to upward migration of cadmium from deeper soil layers induced by precipitation or to exogenous inputs such as the introduction of microbial agents. In July, the CK group exhibited the highest cadmium concentration in leachate at 1.89 mg/L, with an arsenic concentration of 73.1 mg/L, indicating that heavy metals in soil without microbial amendment were more prone to migration with water movement. Notably, the leachate from the PSQ group showed relatively high concentrations of both cadmium and arsenic, suggesting that the genetically engineered strain may have influenced heavy metal mobility by altering soil physicochemical properties such as pH and redox potential. The cadmium concentration in leachate of the PSQ group (2.325 mg/L) was significantly higher than that of the CK group (1.89 mg/L), which may be associated with increased cadmium solubility due to the metabolic activities of the genetically engineered strain. However, the available cadmium concentration in the PSQ group (mean 0.16 mg/kg) decreased by 15.8% compared to that in the CK group (0.19 mg/kg), indicating that the genetically engineered strain reduced cadmium bioavailability through complexation, reduction, or adsorption, thereby immobilizing or transforming cadmium into less bioavailable forms. In the FQ1 group, the original Streptomyces strain promoted cadmium dissolution (leachate cadmium concentration 1.71 mg/L) through organic acid secretion, but did not significantly reduce available cadmium, resulting in lower remediation efficiency compared to the PSQ group.

Precipitation not only influenced soil moisture content but also potentially altered soil redox conditions and nutrient distribution, thereby affecting the composition and function of microbial communities [34]. Microbial community analysis revealed that the Alpha diversity in the experimental groups amended with the genetically engineered strain PSQ and the original Streptomyces strain FQ1 (the PSQ and FQ1 groups) was lower than that in the CK group without microbial amendment, indicating that the introduction of exogenous microorganisms may have inhibited the growth of certain indigenous microorganisms through resource competition or alterations to the rhizosphere environment. The mechanisms underlying the reduction in Alpha diversity may be attributed to competitive effects of the introduced strains: both the PSQ and FQ1 groups exhibited lower Alpha diversity than the CK group, suggesting that the introduction of exogenous strains suppressed indigenous microorganisms via resource competition or secretion of antimicrobial substances. The genetically engineered strain added to the PSQ group, carrying heavy metal resistance genes, possessed a greater survival advantage in the contaminated environment, further compressing the niche space of indigenous microbial communities.

In both the PSQ and FQ1 groups, the abundance of Saccharibacillus increased significantly ($P < 0.05$), suggesting that these strains may decompose polysaccharides in plant root exudates, releasing

low-molecular-weight organic acids that promote cadmium dissolution and plant uptake. Concurrently, the breakdown products of saccharides provide a carbon source for Actinobacteriota (e.g., Ktedonobacter, Gaiellales), forming a synergistic "microbe-microbe-plant" network that immobilizes cadmium through adsorption or biomineralization (e.g., formation of phosphate precipitates), thereby reducing heavy metal migration in leachate [35]. The PSQ group exhibited a relatively high abundance of Actinobacteriota (Figure 3), which may directly reduce cadmium bioavailability by secreting siderophores or reductases (e.g., Cd2+→Cd0), thereby mitigating heavy metal diffusion associated with soil erosion [36].

For the engineered PSQ strain, the combination of enhanced cadmium transformation capacity through genetic editing and modulation of the rhizosphere microbial community establishes a synergistic "immobilization-uptake" mechanism. Genetic modifications (e.g., introduction of heavy metal resistance genes, reductase genes) likely enhance its capacity for cadmium transformation, potentially through the secretion of specific enzymes (e.g., cadmium reductase) that directly reduce cadmium toxicity. Concurrently, the PSQ strain selectively enriches functional microbial groups (e.g., cadmium-tolerant bacteria, saccharolytic bacteria) by modulating the rhizosphere environment (e.g., pH, redox potential), further facilitating the immobilization or transformation of heavy metals.

3.4.3. Plant-Microbial System Analysis

The high cadmium enrichment capacity of Acalypha australis and Cyperus iria (BCF > 7) was closely associated with the saccharolytic and heavy metal immobilization functions of the microbial community, indicating that microorganisms indirectly enhance phytoremediation potential by optimizing the rhizosphere environment, thereby establishing a plant-microbe interactive system. Furthermore, plant uptake capacity was enhanced following the addition of microbial agents. The mean bioconcentration factor (BCF) for cadmium and arsenic in plants in the PSQ group was higher than those in the CK and FQ1 groups, suggesting that the genetically engineered strain may improve remediation efficiency by promoting plant uptake. Notably, Acalypha australis and Cyperus iria exhibited a high cadmium enrichment capacity (BCF > 7), which was closely linked to the saccharolytic and heavy metal immobilization functions of the microbial community. By optimizing the rhizosphere environment, the microorganisms indirectly enhanced the phytoremediation potential of the plants.

4. SUMMARY

This study was conducted as a pot experiment, in which three treatment groups and a control group were established to compare the remediation effects of a genetically engineered strain, a native strain isolated from mine tailings, and a blank control, in combination with plants, on cadmium-contaminated soil. The key experimental results are as follows: After the experiment, the total cadmium concentration in the soil increased slightly (likely due to precipitation-induced leaching), whereas the concentrations of available cadmium (reduced by 15%–30%) and available arsenic (reduced by 10%–40%) decreased significantly, indicating that microorganisms may have reduced heavy metal bioavailability through immobilization or transformation. Among the treatments, the PSQ group exhibited the greatest reduction in available cadmium and arsenic, suggesting that the genetically engineered strain was more effective in suppressing heavy metal activity than the original strain. Plants such as Acalypha australis (with a BCF of up to 11.1) and Cyperus iria (with a BCF of up to 7.8) demonstrated high phytoextraction potential. Regarding microbial assistance, the mean bioconcentration factors (BCF) for cadmium and arsenic in plants were higher in the PSQ group than in the CK and FQ1 groups (e.g., mean cadmium BCF: 6.2 in PSQ vs. 4.9 in CK), suggesting that the genetically engineered strain may enhance remediation efficiency by promoting plant uptake. In terms of soil microbial community diversity, the PSQ and FQ1 groups exhibited lower Alpha diversity than the CK group, with significant differences within groups. A key microbial group, Saccharibacillus, showed significantly increased abundance in the PSQ group ($P < 0.05$), and its saccharolytic capacity

may be associated with enhanced plant-microbe interactions, indirectly facilitating heavy metal immobilization or transformation. Precipitation-induced soil erosion and heavy metal migration were important factors contributing to changes in soil heavy metal concentrations. Notably, the high heavy metal concentrations observed in leachate indicated that precipitation exerted a considerable impact on deeper soil layers and groundwater. The regulatory role of the microbial community, by modifying the rhizosphere environment, reduced heavy metal bioavailability and mitigated the risk of heavy metal migration.

This study validated the potential of the genetically engineered Streptomyces strain PSQ for the remediation of cadmium-contaminated soil through a pot experiment. Strain PSQ significantly reduced the concentrations of available cadmium and arsenic in soil, enhanced the heavy metal accumulation capacity of plants, and formed a synergistic remediation system by regulating the rhizosphere microbial community structure. The combined use of plants such as Acalypha australis and Cyperus iria with microorganisms can achieve a balance between short-term remediation efficiency and long-term soil health. Future efforts should focus on further optimizing remediation strategies and improving remediation efficacy for arsenic contamination through long-term experiments and multi-omics analyses.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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