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Effects of *Acinetobacter johnsonii* and *Rhodotorula mucilaginosa* on the migration and transformation of antimony in soil-pak choi systems

Linqiang Tian¹ & Jiuqin Xian¹, Kun Liu¹, Qian Wang¹, Shixue Mei¹, Aijiang Yang¹,
Wanping Bian², Aping Niu^{1*}

& These authors contributed equally to this work and should be considered co-first authors.

¹ College of Resources and Environmental Engineering, Guizhou Karst Environmental Ecosystems Observation and Research Station, Ministry of Education, Key Laboratory of Karst Georesources and Environment, Ministry of Education, Guizhou University, Guiyang 550025, China.

² Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing, 400714, China.

*Corresponding author. E-mail: apniu@gzu.edu.cn

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Abstract: Soil antimony (Sb) contamination poses a serious threat to the safe production of leafy vegetables and to human health. In this study, two Sb-resistant strains, *Acinetobacter johnsonii* and *Rhodotorula mucilaginosa*, were evaluated for their remediation potential in Sb-contaminated soil using pot experiments with pak choi (*Brassica chinensis* L.) under aerobic conditions. The results demonstrated that both strains significantly increased pak choi biomass, root activity, and photosynthetic pigment content through plant growth-promoting traits such as nitrogen fixation and phosphate solubilization. More importantly, they reduced Sb accumulation in pak choi by promoting the formation of residual and Fe-Mn oxide-bound Sb fractions while inhibiting the formation of humic acid-bound Sb. Notably, *R. mucilaginosa* outperformed *A. johnsonii* in both growth promotion and Sb immobilization. The superior performance of *R. mucilaginosa* is attributed to its unique ability to restructure the rhizosphere fungal community, enriching beneficial taxa such as *Mortierella* and *Pichia*, thereby synergistically enhancing plant resilience to Sb stress. This study provides new insights into the differential mechanisms of bacterial versus fungal remediation of Sb-contaminated soils under aerobic conditions and offers a theoretical basis for developing targeted microbial remediation strategies. The modified membrane exhibits an improved permeability of 54.4% and an average pore size of 50 nm. The ceramic membrane shows promising removal efficiency of Texas dye. Reusability tests of the manufactured membrane demonstrate stable membrane performance over five continuous filtration cycles. The membranes are cleaned by backwashing, and removal tests are conducted for further nine cycles. The membrane maintains high removal efficiency of Texas dye, as measured in chemical oxygen demand (COD), as well as hardness, during the first five cycles. This research highlights the potential of ceramic membranes for efficient, low-pressure dye removal and wastewater treatment, with enhanced durability and reusability.

Introduction

Antimony (Sb) is a toxic metalloid extensively used in the manufacturing of industrial products and has become the ninth most mined metal globally. However, Sb concentrations in soil and water bodies have increased due to natural processes and human activities such as ore mining and smelting (Li et al., 2026). Studies have shown significantly elevated Sb levels in the soil of mining areas, smelters, and shooting ranges. For instance, Sb concentrations in mining areas and surrounding soils often reach hundreds of times higher than background levels (He et al., 2025). Even agricultural and forest soils

are affected by Sb contamination. Sb exhibits biotoxicity and carcinogenicity and tends to accumulate in crops. Sb contamination in farmland soil can inhibit crop growth and development (Tang et al., 2025) and pose risks to human health through the food chain, potentially inducing severe diseases such as liver disorders, cardiovascular diseases, respiratory illnesses, and cancer (Roldán et al., 2025). Therefore, it is of great significance to restore Sb-contaminated farmland.

Traditional remediation techniques for heavy metals, including physical and chemical methods, are often costly, labor-intensive, and may cause secondary pollution (Senila

et al., 2026). In contrast, bioremediation technologies have garnered widespread attention due to their low cost, operational simplicity, environmental compatibility, and strong sustainability (Ogunsola et al., 2025). Among these, microorganism-based remediation strategies have demonstrated promising application prospects in heavy metal pollution control in recent years. Microorganisms can mediate the transformation and immobilization of heavy metals through various mechanisms, such as biosorption, bioprecipitation, redox reactions, and extracellular complexation, thereby reducing their bioavailability and toxicity to plants (Pradhan et al., 2025). Furthermore, certain functional microorganisms can enhance plant growth under heavy metal stress, promoting the sustainable development of agricultural production.

Acinetobacter johnsonii, belonging to the genus *Acinetobacter*, is a common Gram-negative bacterium widely distributed in soil, water, and on plant surfaces. It can secrete various extracellular enzymes and organic acids, and possess nitrogen-fixing and phosphate-solubilizing capabilities, thereby promoting plant growth (Timková et al., 2023). *Rhodotorula mucilaginosa* (abbreviated as *R. mucilaginosa*), a unicellular eukaryote of the genus *Rhodotorula*, possesses remarkable environmental adaptability and is ubiquitously present in diverse ecosystems. It features a complex cell wall structure rich in functional groups, which contributes to its resistance to heavy metal stress (Zhang et al., 2024). Moreover, *R. mucilaginosa* can synthesize antioxidants that assist plants in scavenging reactive oxygen species (ROS) induced by heavy metals, thereby alleviating oxidative damage. Previous studies have shown that these two microorganisms exhibit promising remediation potential for heavy metal pollution, such as lead, cadmium, and arsenic (Angeles de Paz et al., 2023; Timková et al., 2023). However, research on their potential for Sb pollution remediation remains limited, necessitating further exploration of their practical remediation efficacy and underlying mechanisms for the remediation of Sb-contaminated soils.

The toxicity, mobility, and bioavailability of Sb in soil are closely related to environmental conditions, which affect the phytotoxicity and accumulation in plants. Among all various environmental factors, anaerobic and aerobic conditions have different effects on the Sb phytotoxicity. However, most previous studies have mainly focused on Sb accumulation in rice and the use of exogenous microorganisms to remediate Sb accumulation in rice, which is typically grown under anaerobic conditions (Long et al., 2023). There remains a lack of research on the use of exogenous microorganisms to remediate Sb accumulation in crops under aerobic conditions. Leafy vegetables are considered high risk crops for Sb accumulation. Pak choi (*Brassica campestris* L. ssp. *chinensis*) is one of the most common leafy vegetables, with a short growth cycle, and is typically grown under aerobic conditions. The Sb enrichment coefficient of cabbage is 0.01-0.1, which indicates the Sb absorption capacity of pak choi. To understand the environmental behavior and accumulation mechanism of Sb in soil-crops systems under aerobic conditions, pak choi, which is widely cultivated in China, is considered an ideal indicator vegetable for assessing Sb migration and transformation in soil-crops systems under aerobic conditions (Chen et al., 2025).

In our previous study, *A. johnsonii* and *R. mucilaginosa*, which exhibited high Sb-resistance, were screened from Sb-

containing wastewater, and both strains were able to transform Sb(III) into Sb_2O_3 (Shi et al., 2025; Yang et al., 2024). They were used as functional microorganisms to investigate the bioavailability of Sb in soil-pak choi systems under aerobic conditions and their potential for remediating Sb-contaminated farmland. A comprehensive analysis was conducted to evaluate the remediation capabilities of these two microorganisms in Sb-contaminated farmland through indoor pak choi pot experiments. Different microbial treatment groups were established, and the growth indicators and Sb accumulation of pak choi were measured. The plant growth-promoting mechanisms were evaluated by measuring nutritional and growth-promoting indicators. The Sb accumulation mechanism was investigated by measuring Sb speciation and contents, as well as the microbial community structure, in the rhizosphere soil. This study aims to evaluate the remediation effects of *A. johnsonii* and *R. mucilaginosa* in an antimony-contaminated soil-pak choi system and to test the following hypotheses: both strains promote pak choi growth through plant growth-promoting traits such as nitrogen fixation and phosphate solubilization, while reducing antimony accumulation in pak choi by increasing the proportions of iron-manganese bound and residual antimony fractions in the soil. Moreover, *R. mucilaginosa* exhibits superior growth-promoting and antimony-immobilizing capabilities due to its ability to reshape the rhizosphere fungal community and enrich beneficial fungi such as *Mortierella* and *Pichia*.

Materials and methods

Experimental materials and strains

The pak choi variety selected was “Yellow Heart Baby Cabbage” (*Brassica chinensis* L.), characterized by its short growth cycle and strong cold tolerance. For the pot experiment, antimony (Sb) was supplied using potassium antimony tartrate ($C_4H_4KO_7Sb \cdot 1/2H_2O$) with a purity of $\geq 99.0\%$, as the chemical source of Sb (Chongqing Chuandong Chemical Group Co., Ltd., Chongqing, China).

A. johnsonii was isolated from the bottom sediment of Lengshuigou Reservoir (107.86°E, 25.97°N) in Dushan County, Guizhou Province, by our research group, while *R. mucilaginosa* was obtained from antimony mine wastewater at the outlet of Dongfeng Antimony Mine (107.73°E, 25.75°N) in Dushan County, Guizhou Province. Both strains were identified as *A. johnsonii* and *R. mucilaginosa* based on their 16S rDNA sequences. All reagents used in this study were prepared with deionized water provided by EREE deionized water equipment (Shenzhen Yiliyuan Water Treatment Equipment Co., Ltd., Shenzhen, China). The deionized water had a conductivity of $<0.1 \mu S/cm$, and all chemicals used were of analytical grade or higher purity.

Pot experiment of pak choi

Pot experiments were conducted to investigate the effects of *A. johnsonii* and *R. mucilaginosa* on the growth and Sb accumulation of pak choi (*Brassica chinensis* L.) in soils contaminated with different levels of Sb. Plastic pots were used for the pot experiment. The soil was collected from farmland near Shilihetan in Huaxi District, Guiyang City, Guizhou Province. Surface soil (0–20 cm) was collected, naturally air-dried, and then crushed. The physicochemical properties of the original

soil for pak choi cultivation were determined, and the values were as follows: pH, 5.75; organic matter content, $4.76 \text{ mg} \cdot \text{g}^{-1}$; available nitrogen, $62.23 \text{ mg} \cdot \text{kg}^{-1}$; available potassium, $257.7 \text{ mg} \cdot \text{kg}^{-1}$; available phosphorus, $7.13 \text{ mg} \cdot \text{kg}^{-1}$; and background antimony concentration, $15.92 \text{ mg} \cdot \text{kg}^{-1}$. The crushed soil was sieved through a 100-mesh sieve to preliminarily remove impurities. The preprocessed soil was packed into rectangular pots ($49 \text{ cm} \times 18 \text{ cm} \times 15 \text{ cm}$) at 7 kg per pot. Sb solutions prepared from potassium antimonyl tartrate ($\text{C}_4\text{H}_4\text{KO}_7\text{Sb} \cdot 1/2 \text{ H}_2\text{O}$) were added to the soil and thoroughly mixed to achieve exogenous Sb concentrations of 10, 50, and 100 mg/kg . Each treatment group had three replicates, and the pots were aged in a greenhouse for 30 days.

The seeds of pak choi were surface-sterilized with 0.5% NaClO solution, followed by three rinses with deionized water. The pots planted with pak choi seed were placed in a greenhouse with a diurnal temperature cycle of $20\text{--}25^\circ\text{C}$ and a relative humidity of 50%. After the pak choi seeds developed into seedlings with complete root systems, seedlings of uniform size and height were selected and transplanted into pots containing Sb-treated soil aged for 30 days. One group was inoculated with *A. johnsonii* (AJ), and another group was inoculated with *R. mucilaginosa* (RM). The group without inoculation with either microorganism was used as the control to investigate the effects of *A. johnsonii* and *R. mucilaginosa* on the growth and Sb accumulation of pak choi under Sb stress, as well as the microbial communities in rhizosphere soil. The pots were watered every two days to maintain 60% soil moisture. At the end of the pot experiment (45 days), the plants were harvested and thoroughly washed with deionized water.

The analysis of the growth indicators of pak choi and physicochemical property of rhizosphere soil

After collection, the plant roots were washed with a $20 \text{ mmol} \cdot \text{L}^{-1}$ Na-EDTA solution to remove metal ions adsorbed on the root surface, followed by thorough rinsing with deionized water and blotting dry with paper towels. Fresh weight, root weight, stem diameter, and length were recorded. A portion of the collected pak choi samples was dried at 70°C to a constant weight and then separated into roots, stems, and leaves. These tissues were ground using an agate mortar, sieved through a 3 mm mesh, and stored in sealed plastic bags for subsequent analysis. The remaining plant samples were used to determine chlorophyll and carotenoid content. The extracted pigments were diluted to 25 ml with 96% ethanol, and the leaf tissues were removed by centrifugation. The absorbance of the extracts was measured at wavelengths of 665 nm, 649 nm, and 470 nm using a UV spectrophotometer (T6 New Century, Beijing), with 96% ethanol used as the blank control. The contents of chlorophyll a, chlorophyll b, and carotenoids were calculated using Equations (1)–(3). The sum of chlorophyll a and chlorophyll b represented the total chlorophyll content. In the equations, C_a denotes chlorophyll a, C_b denotes chlorophyll b, and C_c denotes carotenoids:

$$C_a = 13.95 \times A_{665} - 6.88 \times A_{649} \quad (1)$$

$$C_b = 24.96 \times A_{649} - 7.32 \times A_{665} \quad (2)$$

$$C_c = \frac{1000 \times A_{470} - 2.05 \times C_a - 114.8 \times C_b}{245} \quad (3)$$

After harvesting the pak choi, soil samples around the root system were collected using the quartering method. The collected soil samples were separated from residual roots. Approximately 0.5 g of soil was used for microbial community analysis. The remaining soil samples were air-dried, ground with an agate mortar, sieved through a 100-mesh sieve, and stored in sealed plastic bags for further analysis. Rhizosphere soil pH was measured using a CO_2 -free distilled water extraction method and readings were taken with a calibrated pH meter after 30 min of settling following stirring. The contents of available nitrogen (N), available phosphorus (P), available potassium (K), and organic matter in the soil were determined using the alkali hydrolysis diffusion method for available N, the hydrochloric acid-ammonium fluoride method for available P, the NH_4OAc extraction method for available K, and the chromate oxidation method for organic matter, respectively. More details are provided in Text S1.

Plant growth-promoting characteristics of *A. johnsonii* and *R. mucilaginosa*

To determine the plant growth-promoting characteristics of *A. johnsonii* and *R. mucilaginosa*, their abilities to produce ammonia, fix nitrogen, solubilize phosphorus, and produce siderophores were assessed. **Ammonia production ability:** Under aseptic conditions, the activated strain was inoculated at a 2% (v/v) ratio into 50 mL of peptone water medium (the control group received no bacterial suspension). The cultures were incubated at $28\text{--}30^\circ\text{C}$ with shaking at 150–200 rpm for 24–72 hours. A certain amount of bacterial culture was transferred into a clean test tube, and 1–2 drops of Nessler's reagent were slowly added along the tube wall. The color change in the solution was observed. If the interface or the entire solution turned brownish-yellow, it indicated that the strain possessed ammonia-producing ability. **Nitrogen fixation ability test:** The activated bacterial suspension was spot-inoculated onto Ashby's nitrogen-free medium using an inoculation loop or a micropipette and cultured at $28\text{--}30^\circ\text{C}$ for 3–7 days. The formation of colonies on the medium indicated that the strain possessed nitrogen fixation ability. **Phosphate solubilization ability test:** The activated bacterial suspension was spot-inoculated onto Pikovskaya (PKO) medium and cultured at $28\text{--}30^\circ\text{C}$ for 3–7 days. The formation of a transparent halo (phosphorus solubilization zone) around the colonies indicated that the strain possessed phosphate-solubilizing ability. **Siderophore production test:** The activated bacterial strain was inoculated into a low-iron liquid medium and cultured in a shaker at 30°C and 150 rpm for a specified duration. After centrifugation, the supernatant was collected, and Chrome Azurol S (CAS) assay detection solution was added (the control group consisted of sterilized medium without bacterial inoculation). The mixture was left to stand for a specified period, and the color change was observed. If the solution color changed from blue to orange-yellow, it indicated that the strain possessed siderophore-producing ability. The specific experimental methods and medium formulations are provided in Text S2 and Table S1.

Analysis of Sb content in pak choi and soil samples

Approximately 0.5 mg of ground plant sample was placed into a PTFE-sealed digestion vessel and digested with 68%–

70% HNO₃ and 70%–72% HClO₄ (5:1, v/v) at 180°C for 2 hours. Approximately 0.25 g of ground soil was digested with 1 mL of 30% hydrogen peroxide, followed by the addition of 2 mL of hydrochloric acid and 5 mL of nitric acid. The reaction was performed using a microwave digestion system (MARS 6, USA). The samples were heated at 200 °C until the digestion solution was concentrated to 2 mL. The cooled plant and soil digests were then diluted to 50 mL, and 5 mL of the diluted solution was filtered through a 0.22 µm mixed cellulose ester (MCE) membrane filter. The total Sb concentration in the soil and plant sample solutions was determined using an atomic fluorescence spectrophotometer (AFS-9800, Shanghai). The operational parameters were as follows: 5% HCl was used as the carrier solution; the reducing agent was a mixture of 2% KBH₄ and 0.5% NaOH; and the carrier gas and shielding gas flow rates were 300 mL/min and 800 mL/min, respectively.

According to the sequential extraction method, the chemical speciation of Sb in the pak choi soil was determined. The chemical forms of Sb in the rhizosphere soil of pak choi were primarily classified into the following categories: exchangeable (F1), specifically adsorbed (F2), fulvic acid-complexed (F3), humic acid-complexed (F4), poorly crystalline iron-manganese oxide-bound (F5), sulfide-bound (F6), and residual (F7) Sb (Krishnamurti and Naidu, 2003). For each extraction step, 10 mL of the extract was centrifuged at 10,000 r/min for 10 minutes, and the supernatant was filtered through a 0.22 µm membrane filter. The Sb content was then measured using an atomic fluorescence spectrophotometer (AFS-9800, Shanghai).

Analysis of soil microbial community in root zone of pak choi

To investigate the effects of *A. johnsonii* and *R. mucilaginosa* on soil microbial communities, soil samples were collected at the end of the pot experiment. Bacterial and fungal DNA was extracted using the OMEGA E.Z.N.A.TM Mag-Bind Soil DNA Kit according to the manufacturer's instructions. The concentration and quality of the extracted DNA were measured using a Qubit® 4.0 Fluorometer (Q33238, Thermo Fisher). For amplification of bacterial 16S rRNA and fungal ITS genes, the following primers were used for PCR amplification. For bacterial 16S rRNA, the primers were 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC). For fungal ITS, amplification was performed using the primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2R (GCTGCGTTCTTCATCGATGC). Following Sanger sequencing, the 16S amplified fragments and full-length sequences were aligned using the RDP Classifier against reference sequences in the Silva database, while the ITS amplified fragments and full-length sequences were aligned using Blast against reference sequences in the UNITE database. To assess bacterial and fungal diversity and relative abundance, the V3-V4 region of the bacterial 16S rRNA gene and the ITS1-ITS2 region of the fungal ITS gene were sequenced on the Illumina MiSeq platform (Shanghai Majorbio Bio-pharm Technology Co., Ltd.).

Statistical analysis

The experimental results are presented as the mean ± standard deviation of three replicates. Statistical analyses

were performed using GraphPad Prism 9.0 and Origin 2021 software. Data were analyzed using one-way analysis of variance (ANOVA). Differences were considered statistically significant at **p* < 0.05, ***p* < 0.01, and ****p* < 0.001.

Results

Effects of *A. johnsonii* and *R. mucilaginosa* inoculation on the growth and physiology of pak choi

The growth and physiological response of pak choi under antimony (Sb) stress reflect the Sb-induced phytotoxicity. To assess the effects of *A. johnsonii* and *R. mucilaginosa* on pak choi growth, we measured the fresh weight, root weight, stem diameter, and plant height under different Sb stresses. Compared to the control, both microbial treatments promoted the pak choi growth (Fig. 1 A). All inoculations enhanced root development, with the *R. mucilaginosa*-inoculated group exhibiting more robust growth (Fig. 1 A). The fresh weight of pak choi with *A. johnsonii* inoculation increased by 6.74%–168.12% at different Sb concentrations compared to the control groups, while *R. mucilaginosa* inoculation showed a greater increase of 12.05%–198.85%, overall outperforming *A. johnsonii* (Fig. 1 B). For root weight, *A. johnsonii* treatment exhibited a relatively moderate increase (2.00%–2.37%), whereas the *R. mucilaginosa* treatment demonstrated a highly significant enhancement, reaching 92.31%–374.19% (Fig. 1 C). A consistent trend was observed in stem diameter, increasing by 10.08%–20.53% and 15.63%–30.02% in the *A. johnsonii* and *R. mucilaginosa* treatment groups, respectively (Fig. 1 D). Regarding plant height, the *R. mucilaginosa* treatment remained superior to that of *A. johnsonii*, achieving a maximum increase of 25.16% (Fig. 1 E).

Analysis of key physiological indicators demonstrated that both *A. johnsonii* and *R. mucilaginosa* inoculation effectively improved the physiological status of pak choi under Sb stress, while *R. mucilaginosa* treatment generally exhibited more significant enhancement in various indicators (Fig. 2 A–C). Compared to the control groups, root vitality (Fig. 2 A) increased by 27.00%–37.58% in the *A. johnsonii* treatment and by 44.36%–50.47% in the *R. mucilaginosa* treatment, which showed a greater improvement. The change trend in photosynthetic pigment content was similar to that of root vitality. Chlorophyll contents increased by 12.05%–50.79% and 21.50%–68.69% in the *A. johnsonii* and *R. mucilaginosa* groups, respectively (Fig. 2 B), while carotenoid contents increased by 9.38%–35.14% and 21.43%–58.75%, respectively (Fig. 2 C). The effect of *R. mucilaginosa* on photosynthetic pigments was superior to that of *A. johnsonii*.

These data demonstrate that inoculation with both *A. johnsonii* and *R. mucilaginosa* can effectively promote growth and enhance the physiological performance of pak choi under Sb stresses. *R. mucilaginosa*, however, exhibited relative advantages in both growth promotion and physiological enhancement.

Growth-promoting characteristics of *A. johnsonii* and *R. mucilaginosa*

To elucidate the mechanisms by which *A. johnsonii* and *R. mucilaginosa* promote plant growth, we evaluated several key growth-promoting traits of both strains, including ammonia

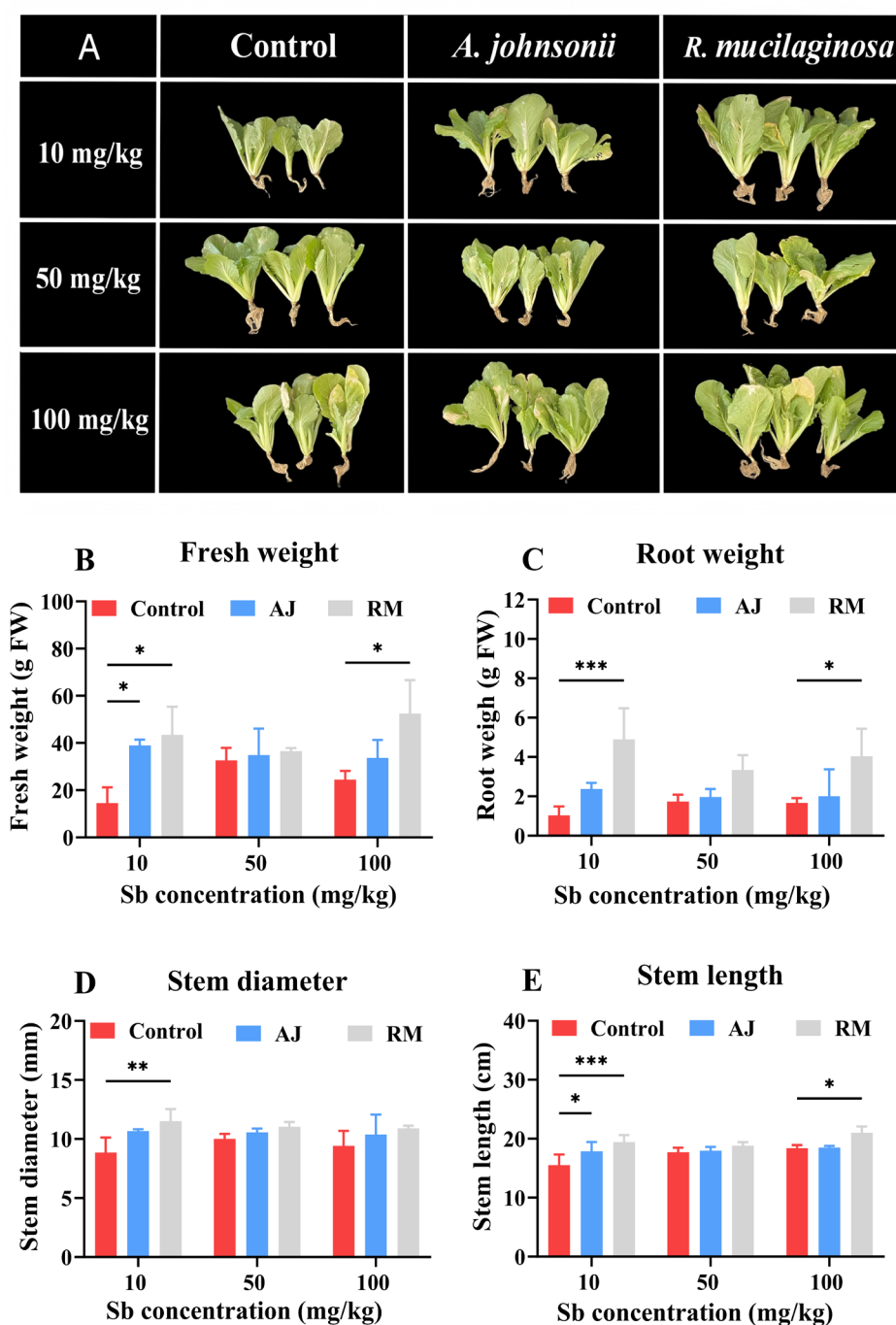


Fig 1. Growth parameters of pak choi. (A) Representative image; (B) Fresh weight; (C) Root weight; (D) Stem diameter; (E) Stem length. (AJ represents vaccination with *A. johnsonii*; RM represents vaccination with *R. Mucilaginosa*).

Error bars represent standard deviations from three independent replicates. Significant differences were calculated as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

production, nitrogen fixation, phosphate solubilization, and siderophore production (Fig. 2 D-G). In the ammonia production test, the solutions inoculated with both *A. johnsonii* and *R. mucilaginosa* turned brownish-yellow, confirming their ammonia-producing capability (Fig. 2 D). Both strains formed distinct colonies on Ashby's nitrogen-free medium, confirming their nitrogen-fixing capabilities (Fig. 2 E). Similarly, transparent halos (phosphorus solubilization zones) formed around the colonies of both *A. johnsonii* and *R. mucilaginosa* on the culture medium (Fig. 2 F), which is attributed to their

decomposition of phosphates, confirming that both strains possess phosphate-solubilizing ability. Furthermore, after being cultured in a low-iron medium with the addition of CAS detection solution, the color of bacterial suspensions with *A. johnsonii* or *R. mucilaginosa* changed from blue to light yellow (Fig. 2 G), indicating that both strains possess the ability to produce siderophores.

Effects of *A. johnsonii* and *R. mucilaginosa* on the physicochemical properties of rhizosphere soil

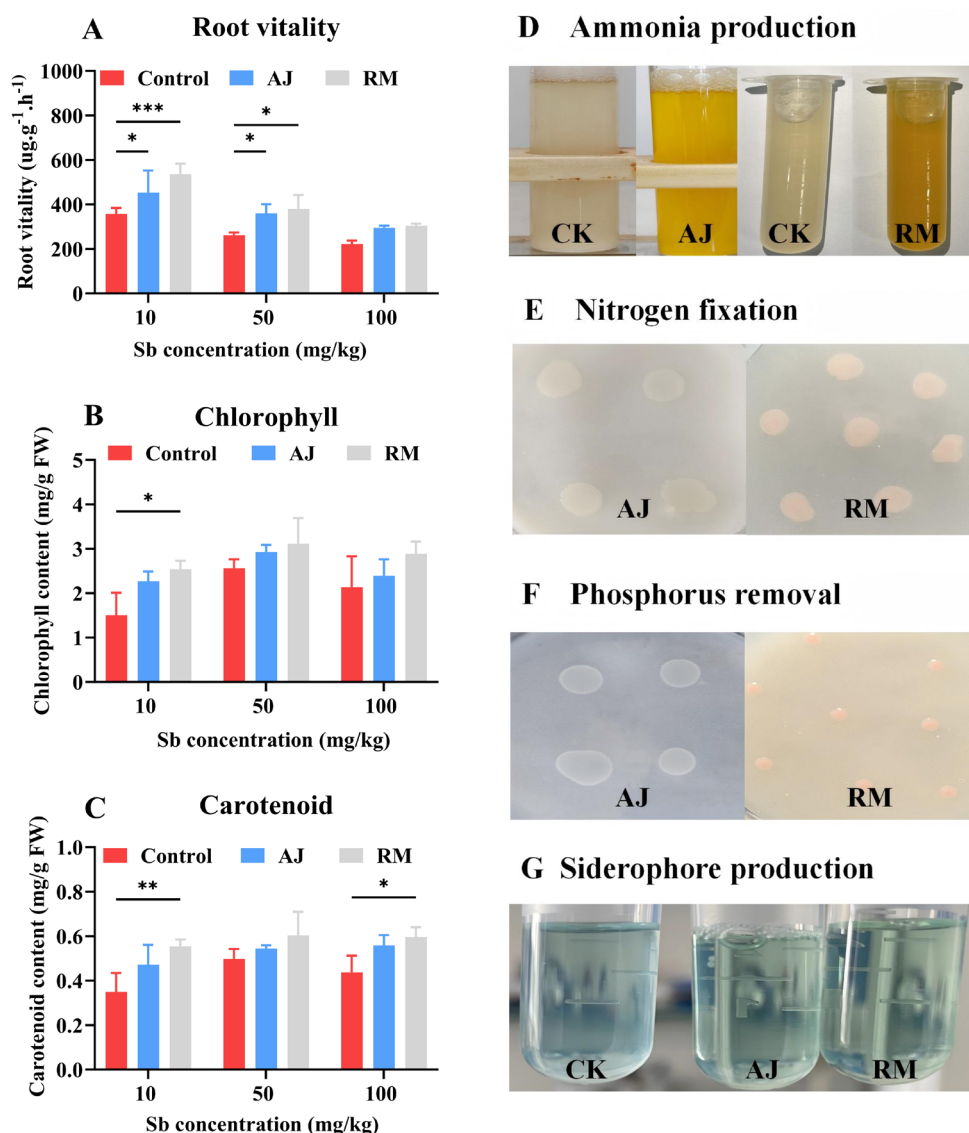


Fig 2. Physiological parameters of pak choi and plant Growth-Promoting Characteristics of *A. johnsonii* and *R. mucilaginosa*: (A) Root vitality; (B) Chlorophyll content; (C) Carotenoid content; (D) Ammonia production capacity determination; (E) Nitrogen fixation capacity determination; (F) Phosphorus solubilisation capacity determination; (G) Determination of iron carrier production ability. (AJ represents vaccination with *A. johnsonii*; RM represents vaccination with *R. mucilaginosa*). Error bars indicate standard deviations from three replicate experiments. Significance levels denoted as * $p < .05$, ** $p < .01$, and *** $p < .001$.

To elucidate the mechanisms underlying plant growth-promotion, we analyzed key physicochemical properties of the rhizosphere soil (Fig. 3). The results showed that inoculations with *A. johnsonii* or *R. mucilaginosa* exerted distinct effects on soil nutrient parameters. Soil pH was stabilized at approximately 6.0 at all treatment groups. The organic matter content in rhizosphere soil increased after *A. johnsonii* or *R. mucilaginosa* inoculations (Fig. 3 A). The *A. johnsonii* treatment group exhibited an increase of 4.09%–7.00%, while the *R. mucilaginosa* treatment showed an increase of 2.78%–7.62%, indicating that microbial inoculation promotes the accumulation of soil organic matter to some extent. Similarly, available nitrogen (Fig. 3 B) increased by 1.75%–11.98% in the *A. johnsonii* group and by 9.19%–19.96% in the *R. mucilaginosa* group compared to the control. This suggests that microbial inoculation can significantly enhance the available nitrogen content in rhizosphere soil, particularly with *R. mucilaginosa*.

In contrast, microbial inoculation significantly reduced the levels of available phosphorus and potassium in rhizosphere soil (Fig. 3 C-D). Available phosphorus (Fig. 3 C) decreased by 9.80%–15.25% and 7.84%–18.64% in the *A. johnsonii* and *R. mucilaginosa* treatment groups, respectively. A more pronounced reduction was observed for available potassium, which decreased by 53.61%–66.25% and 15.33%–44.24% in the *A. johnsonii* and *R. mucilaginosa* groups, respectively (Fig. 3 D). These results demonstrate that both *A. johnsonii* and *R. mucilaginosa* enhanced soil organic matter and available nitrogen but significantly reduced available phosphorus and potassium in the rhizosphere soil of pak choi.

Antimony content in pak choi and rhizosphere soil and antimony fractionation in rhizosphere soil

To investigate Sb migration and transformation in soil-plant system under aerobic conditions, we quantified Sb

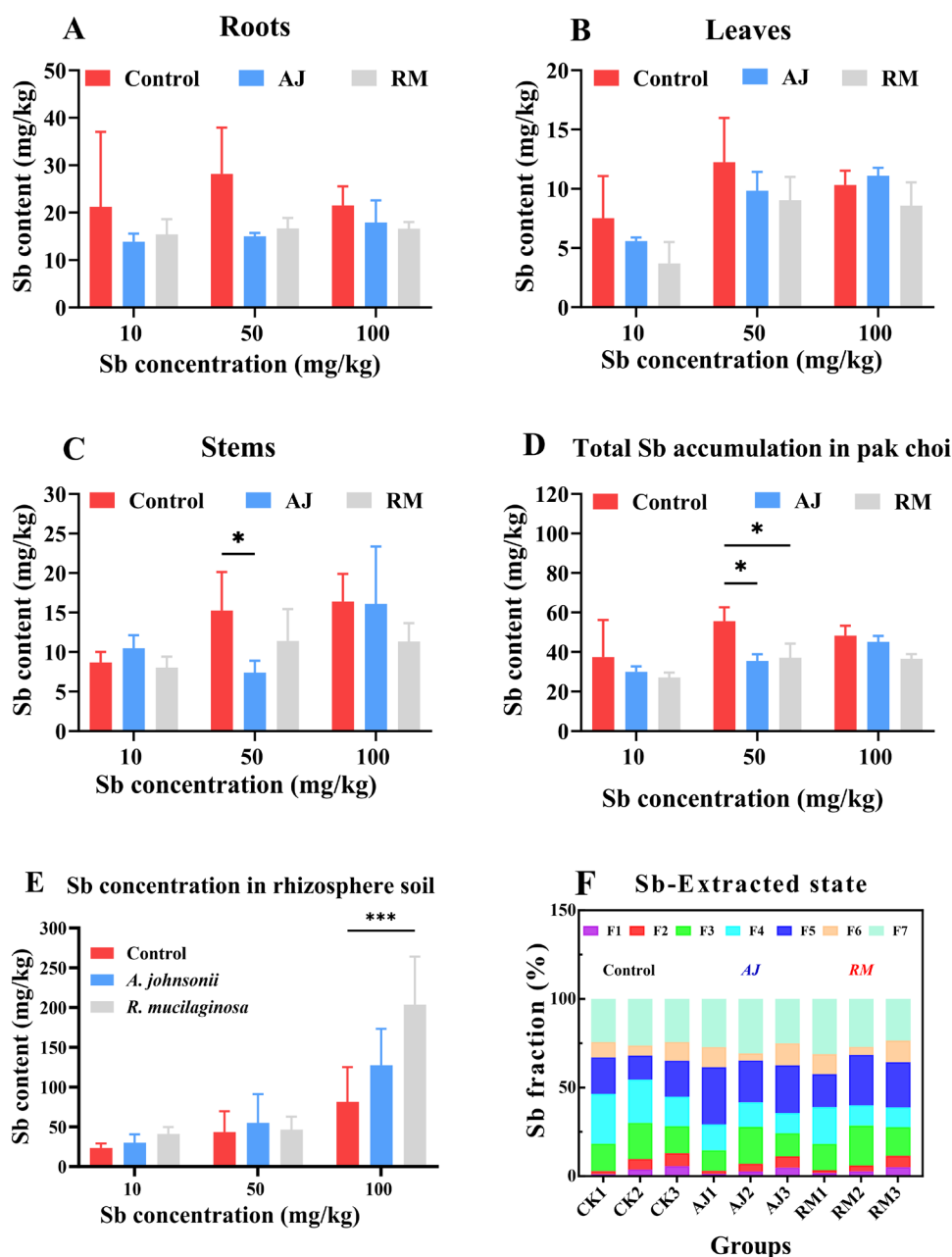


Fig 3. Illustrates the physicochemical characteristics of the soil with pak choi cultivation. (A) Organic matter content, (B) Available nitrogen, (C) Available phosphorus, and (D) Available potassium. (AJ represents vaccination with *A. johnsonii*; RM represents vaccination with *R. Mucilaginosa*). Error bars indicate standard deviations based on three parallel experiments. Significance levels were denoted as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

contents in various pak choi tissues and the rhizosphere soil, and analyzed Sb speciation in rhizosphere soil (Fig. 4). In the roots, Sb content decreased by 16.65%–46.72% and 22.88%–40.75% in the *A. johnsonii* and *R. mucilaginosa* groups, respectively (Fig. 4 A). In the leaves (Fig. 4 B), Sb content decreased by 19.69% and 25.65% in the *A. johnsonii* groups with 10 and 50 mg/kg Sb treatments, respectively. In contrast, *R. mucilaginosa* reduced leaf Sb contents in all Sb treatment groups, with decreases of 17.06% - 40.75%. A similar trend was observed in the stems (Fig. 4 C). Under the 50 and 100 mg/kg Sb treatments, the Sb content in the *A. johnsonii* groups decreased by 29.55% and 1.78% compared to the corresponding control groups, respectively. Whereas *R. mucilaginosa* reduced stem Sb contents across all Sb treatment

groups, with reductions of 7.36% - 30.85%. The overall Sb enrichment pattern in pak choi tissues was: root > leaf > stem. On the whole, microbial inoculation significantly reduced total Sb content in pak choi plants (Fig. 4 D), particularly under the 50 mg/kg Sb stress, with reduction of 36.07% and 33.26% for the *A. johnsonii* and *R. mucilaginosa* treatments, respectively. These results demonstrate that both *A. johnsonii* and *R. mucilaginosa* effectively inhibited Sb uptake and accumulation in pak choi tissues. The *R. mucilaginosa* treatment exhibited relatively obvious and stable reduction on Sb accumulation in tissues at the tested Sb concentrations.

The Sb concentration in the rhizosphere soil of pak choi increased with elevated exogenous Sb levels (Fig. 4 E). Sb concentrations in the rhizosphere soil with *A. johnsonii* or

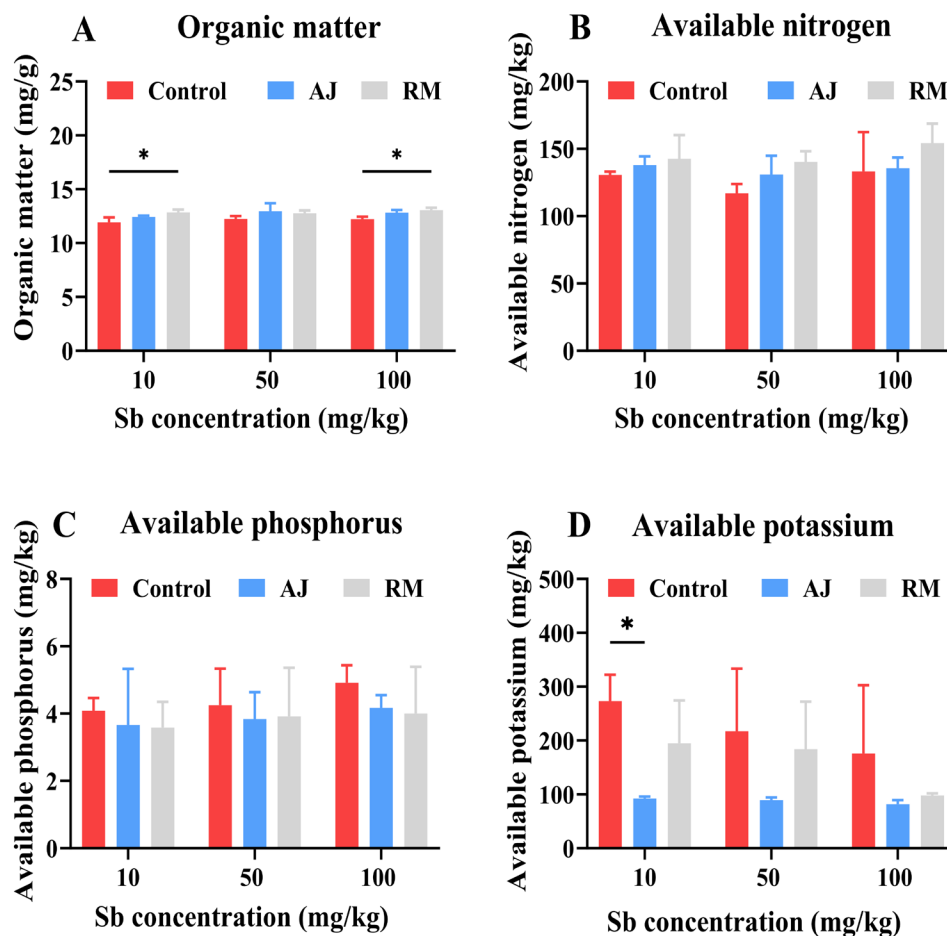


Fig 4. Accumulation content and chemical forms of Antimony in pak choi tissues and rhizosphere soil. (A) root; (B) leaves; (C) stem; (D) total Sb content; (E) rhizosphere soil; (F) chemical forms of Sb in the rhizosphere soil (F1 is the exchangeable form, F2 is the specific adsorption form, F3 is the fulvic acid-bound form, F4 is the humic acid-bound form, F5 is the weakly crystalline iron and manganese oxide-bound form, F6 is the sulfide-bound form, and F7 is the residual form). All the three groups, CK (control group), AJ and RM, were treated with 10, 50, and 100 mg/kg Sb respectively. Error bars indicate the standard deviation of three parallel experiments. Significant differences are calculated as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

R. mucilaginosa were significantly higher than those in the control groups. In particular, Sb contents in the rhizosphere soil with *A. johnsonii* and *R. mucilaginosa* reached 127.61 and 203.75 mg/kg in the 100 mg/kg groups, with increases of 56.93% and 150.56%, respectively, which were much higher than the average Sb content in the soil.

In this study, sequential extraction was used to analyze the fractionation of Sb in the rhizosphere soil after pak choi harvest. In all treatment groups, the residual fraction (F7), weakly crystalline Fe-Mn oxide-bound fraction (F5), and humic acid-bound fraction (F4) predominated in the Sb speciation distribution (Fig. 4 F). Compared with the control groups, *A. johnsonii* and *R. mucilaginosa* inoculation significantly promoted the formation of Fe-Mn oxide-bound Sb and inhibited the formation of humic acid-bound Sb. The F5 fraction increased by 32.65%–73.73% and 24.74%–110.48% in the *A. johnsonii* and *R. mucilaginosa* groups, respectively. The F4 (humic acid-bound form) decreased by 31.02%–47.99% and 25.83%–53.26% after *A. johnsonii* and *R. mucilaginosa* inoculation, respectively. Meanwhile, the residual Sb (F7) in the *A. johnsonii* and *R. mucilaginosa* groups increased by

3.17%–24.69% and 2.74%–28.52%, respectively, compared to the control groups.

The microbial community composition in rhizosphere soil of pak choi

The rhizosphere microflora play a crucial role in modulating crop growth and heavy metal accumulation. Therefore, the microbial community in the rhizosphere was detected and analyzed through high-throughput sequencing. At the bacterial phylum level, *Pseudomonadota* and *Bacteroidota* were dominant in all groups, with their total relative abundance exceeding 50% (Fig. 5 A). Inoculation with *A. johnsonii* did not alter their combined abundance, whereas *R. mucilaginosa* increased the total relative abundance of the two phyla under different Sb stresses. At the bacterial genus level (Fig. 5 C), the dominant genera in all groups were *Sphingomonas* and *Flavisolibacter*. Neither *A. johnsonii* nor *R. mucilaginosa* inoculation had a significant effect on the total relative abundance of the two genera. However, *R. mucilaginosa* inoculation reduced bacterial diversity at the genus level.

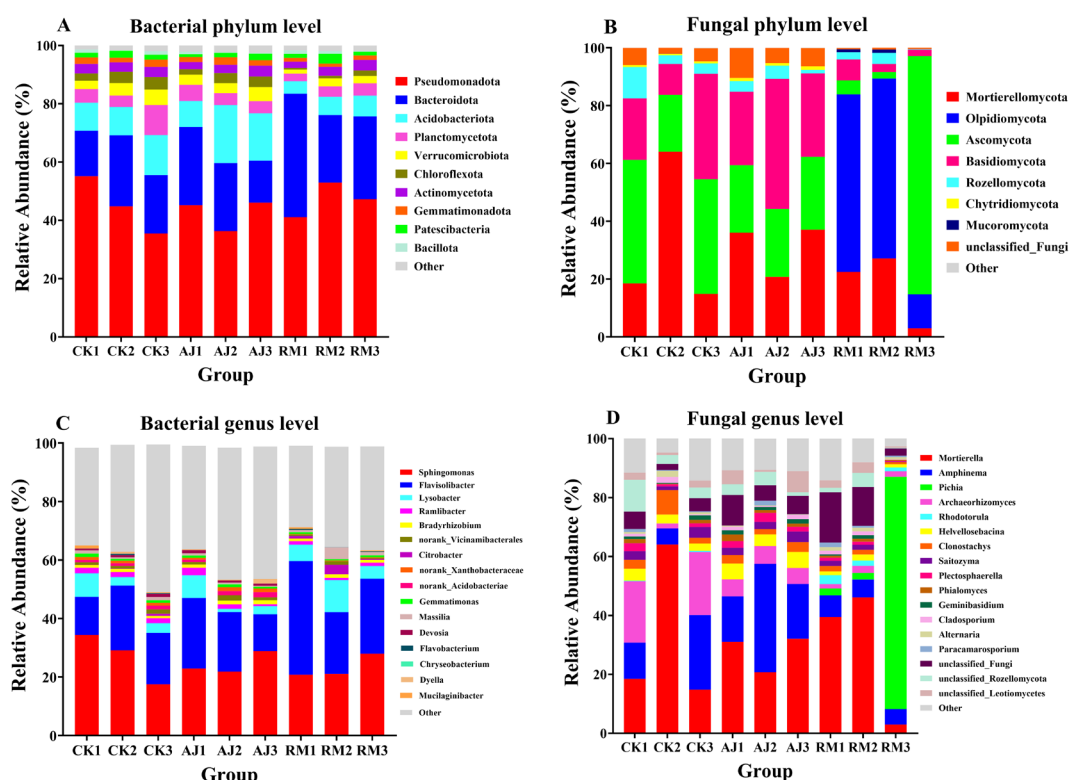


Fig 5. Taxonomic composition of bacterial and fungal communities in the rhizosphere soil of pak choi inoculated with *A. johnsonii* and *R. mucilaginosa*. (A) Bacterial phylum level; (B) Fungal phylum level; (C) Bacterial genus level; (D) Fungal genus level. (All the three groups, CK (control group), AJ and RM, were treated with 10, 50, and 100 mg/kg Sb respectively.)

At the fungal phylum level (Fig. 5 B), *Mortierellomycota*, *Ascomycota* and *Basidiomycota* were dominant in the control and *A. johnsonii*-inoculated groups, with *A. johnsonii* inoculation showing no significant impact on their total relative abundance. In contrast, the fungal community with *R. mucilaginosa* inoculation was dominated by *Olpidiomyces* or *Ascomycota*, accounting for over 60% of the abundance. At the fungal genus level (Fig. 5 D), *Mortierella*, *Amphinema* and *Archaeorhizomyces* were the main genera in the control and *A. johnsonii*-inoculated groups, and *A. johnsonii* inoculation had no obvious effect on the total relative abundance of dominant fungi. In contrast, *Mortierella* or *Pichia* were the dominant fungi in the group with *R. mucilaginosa* inoculation, and their relative abundance accounted for over 60%. Furthermore, *R. mucilaginosa* inoculation decreased fungal community diversity at the genus level.

The observation demonstrated that *A. johnsonii* inoculation had no significant effects on microbial communities, while *R. mucilaginosa* altered the community structure with its effect being especially significant for the fungal community. Notably, *R. mucilaginosa* inoculation reduced the abundance of certain potential pathogenic fungi, such as some *Clonostachys* and *Plectosphaerella* species, thereby further optimizing the microbial community structure. This helps mitigate the risk of pathogen infection in plants under heavy metal stress and enhances their overall resilience.

Distribution patterns and influence factors of rhizosphere microbial community

In order to study the regulatory effects of exogenous microorganisms and environmental factors on the rhizosphere

microbial community, Principal Co-ordinates Analysis (PCoA) and Redundancy Analysis (RDA) were performed (Fig. 6). PCoA results revealed that inoculation with *A. johnsonii* induced a certain alteration in bacterial community structure, while exerting a relatively weaker influence on the fungal community. In contrast, *R. mucilaginosa* inoculation exerted a stronger influence on the structures of bacterial and fungal communities compared with *A. johnsonii* (Fig. 6 A-B). The influence of *R. mucilaginosa* on the fungal community was more obvious than that on the bacterial community.

RDA conducted using the ChiPlot online analysis platform further indicated a significant correlation between changes in rhizosphere nutrients and growth-promoting indicators of pak choi under the two strain treatments. RDA1 of bacteria and fungi in the groups with *A. johnsonii* inoculation explained 59.85% and 67.83% of the community variation, respectively (Fig. 6 C-D). The environmental factors of available nitrogen (HN) and available potassium (AK) showed significant associations with the microbial community variation of bacteria and fungi in the groups with *A. johnsonii* inoculation, respectively. For the groups with *R. mucilaginosa* inoculation, RDA1 accounted for 66.46% and 70.47% of the variation (Fig. 6 E-F). pH and HN were the main influencing factors for bacterial community variation, while available phosphorus (AP) and Sb were the main influencing factors for fungal community variation.

Furthermore, the explained variance of RDA2 was higher (27.45%) with *R. mucilaginosa* inoculation than that in the *A. johnsonii* treatments, indicating a stronger regulatory effect of *R. mucilaginosa* on the microbial community and a closer association of environmental factors with the

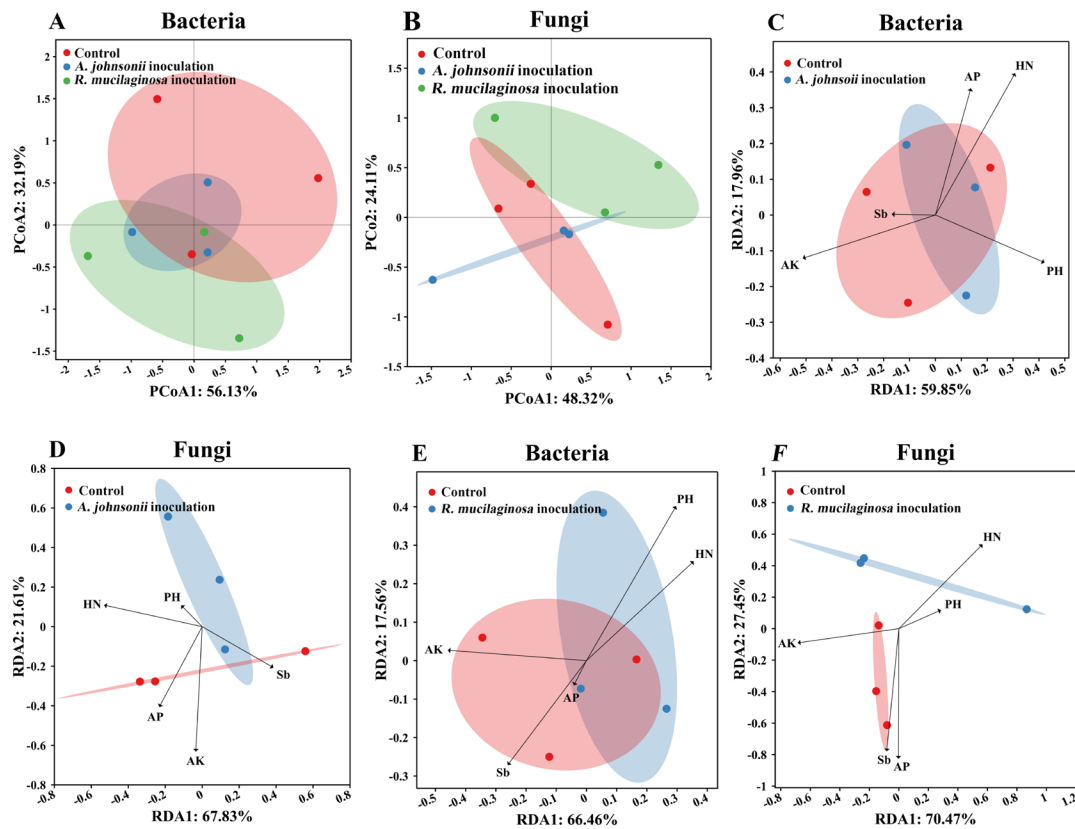


Fig 6. Analysis of the effects of *A. johnsonii* and *R. mucilaginosa* treatments on the microbial community in the rhizosphere soil of pak choi. (A, B) Principal coordinate analysis of bacterial and fungal communities; (C, D) Redundancy analysis of bacterial and fungal communities and soil physicochemical factors under *A. johnsonii* treatment; (E, F) Redundancy analysis of bacterial and fungal communities and soil physicochemical factors under *R. mucilaginosa* treatment.

microbial community in the group with *R. mucilaginosa*. The integrated PCoA and RDA results suggest that *R. mucilaginosa* exerts a more profound influence on microbial communities through synergistic interactions with environmental factors, demonstrating a particularly distinct advantage in restructuring the fungal community.

Discussion

The effectiveness of microbial remediation for heavy metal-contaminated soils varies significantly, depending on the biological characteristics, remediation mechanisms, and environmental adaptability of the microorganisms involved. Therefore, even microorganisms with “identical” remediation mechanisms might exhibit different effectiveness. Currently, research on the remediation of Sb contamination by bacteria and fungi remains limited, and the underlying mechanisms remain poorly understood. In our previous study, both *A. johnsonii* and *R. mucilaginosa* exhibited the ability to mineralize Sb(III) into Sb_2O_3 , with *R. mucilaginosa* exhibiting higher Sb(III) resistance and mineralization efficiency than *A. johnsonii* (Shi et al., 2025; Yang et al., 2024). This study systematically evaluated the effects of these two strains on pak choi growth, Sb accumulation in plants, Sb speciation in soil, soil physicochemical properties, and rhizosphere microbial community.

The results demonstrated that both microorganisms can promote pak choi growth under Sb stress and effectively reduce

Sb accumulation in plant tissues. However, *R. mucilaginosa* is superior to *A. johnsonii* in both promoting plant growth and reducing Sb accumulation. These findings are consistent with recent trends in microbe-assisted phytoremediation research, as multiple microorganisms have been shown to alleviate antimony stress by promoting plant growth and altering the speciation of antimony in soil (Wu et al., 2025). However, the differences in efficiency among various microorganisms also reflect a high degree of strain-specific dependence of remediation outcomes. For example, arbuscular mycorrhizal fungi (AMF) reduced antimony uptake and translocation in flax by 47% (Zrig et al., 2025), while the combination of the antimony-resistant plant growth-promoting bacterium *Bacillus* sp. D2 with Fe^{2+} reduced antimony translocation in rice stems by 67.6% (Wu et al., 2025). In comparison, *R. mucilaginosa*, which performed better in this study, resulted in even lower antimony accumulation in plant tissues, demonstrating an efficacy comparable to these reported high-efficiency remediation strategies.

Nitrogen serves as a fundamental element for chlorophyll synthesis; therefore, its availability directly influences chlorophyll content and photosynthetic efficiency. Inoculation with *A. johnsonii* or *R. mucilaginosa* significantly increased the available nitrogen content in rhizosphere soil and effectively elevated the leaf chlorophyll content. The effect was more pronounced in the *R. mucilaginosa* treatment, indicating its capacity to enhance nitrogen uptake in pak choi compared to *A.*

johnsonii. This difference is further corroborated in the study by Paul et al. (2029), in which *R. mucilaginosa* JGTA-S1 can form a tripartite interaction system by symbiotically interacting with the endophytic nitrogen-fixing bacterium *Pseudomonas stutzeri*, resulting in sustained expression of nitrogenase genes even under nitrogen-sufficient conditions. Consequently, it activates nitrogen metabolism-related transcription factors within 24 hours of colonization, providing a rapid nitrogen supply to rice (Paul et al., 2019). In contrast, although *A. johnsonii* also possesses nitrogen solubilization and heterotrophic nitrification capabilities (Jangra et al., 2024), its nitrogenase function is readily inhibited under high-nitrogen conditions. Therefore, this mechanistic difference may be a potential reason for the superior plant growth-promoting effect of *R. mucilaginosa* observed in this study.

Appropriately increasing the nutrients can promote root growth and nutrient absorption. Consistent with this, inoculation with either *A. johnsonii* or *R. mucilaginosa* distinctly decreased the contents of available phosphorus and potassium in soil, suggesting enhanced uptake of these elements by pak choi. Root vitality refers to the ability of nutrients uptake and environmental responsiveness and is a key determinant of plant growth and yield. Both *A. johnsonii* and *R. mucilaginosa* inoculations distinctly enhanced root vitality, but the effect of *R. mucilaginosa* inoculation was more pronounced. This result is highly consistent with existing studies on PGPR-mediated root system regulation. For example, Naveen and Balachandar et al. (2025) found that *Aeromonas hydrophila* QS74, owing to its abundant extracellular polymeric substance secretion capability, significantly enhances soil organic carbon, microbial activity, and aggregate stability, and improves the nutrient acquisition capacity of subsequent crops through a positive feedback mechanism of “soil conditioning–root response” (Naveen and Balachandar, 2025). Accordingly, it is further inferred that the mechanisms by which *A. johnsonii* and *R. mucilaginosa* enhance root activity are multifaceted, encompassing volatile signal transduction, rhizosphere microenvironment remodeling, and feedback regulation of the soil–plant system.

Both strains exhibit significant plant growth-promoting traits, including ammonia production, nitrogen fixation, phosphate solubilization, and siderophore synthesis. On this basis, *A. johnsonii* also produces indole compounds such as indole-3-lactic acid (ILA), and its growth-promoting chemical composition has been confirmed by mass spectrometry (Bhattacharya et al., 2024). Meanwhile, *R. mucilaginosa* RSRod01 possesses combined abilities of siderophore production, extracellular enzyme secretion, and heavy metal tolerance, and can maintain its growth-promoting activity under a salt concentration of 4% (Rahal et al., 2023). Thus, the aforementioned multiple growth-promoting traits of the two strains constitute the direct basis for their plant growth-promoting effects.

The toxicity and bioavailability of heavy metals primarily depend on their speciation in the environment. Iron-manganese oxides exhibit a strong adsorbing capacity for Sb(III) and Sb(V); however, the plant availability of these adsorbed species is significantly different. Compared to Sb(III), iron-manganese oxide-bound Sb(V) is difficult to be absorbed by plant roots (Shao et al., 2021). In recent years, research

on microbe-mediated antimony transformation has provided new approaches for reducing antimony bioavailability. For example, *Pseudomonas* sp. QJX-1, isolated from an antimony mining area, can oxidize Sb(III) to Sb(V) through the in situ formation of biogenic manganese oxides (BMO), and only the Sb(V) generated by the oxidation process can be efficiently adsorbed by BMO. The Fe(II) produced from the oxidation further forms FeOOH and mixes with BMO to constitute biogenic iron-manganese oxides (BFMO), achieving the synergistic immobilization of antimony through oxidation and adsorption (Bai et al., 2017). In addition, a team from Huazhong Agricultural University recently identified a bifunctional enzyme, AntO, that simultaneously catalyzes Sb(III) oxidation and H₂O₂ decomposition (Luo et al., 2026), providing direct molecular evidence for the enzymatic regulation of soil antimony speciation by microorganisms.

Under aerobic conditions, Sb in soil mainly exists as Sb(V). Residual Sb has low biological availability and is difficult for plants to absorb. Therefore, increases in the iron-manganese oxide-bound and residual proportions in the rhizosphere soil contribute to reducing Sb absorption by pak choi roots. Humic acid and other dissolved organic matter can significantly increase the solubility and mobility of antimony in soil through complexation and competitive adsorption, thereby raising the risk of crop uptake. Notably, the interaction effects between organic matter from different sources and iron-manganese oxides vary significantly. Straw-derived organic-inorganic composite colloids (SD-Fe/Mn) form a more stable colloidal structure with iron-manganese oxides, resulting in the near-complete stabilization (100%) of water-soluble and carbonate-extractable antimony after 35 days of treatment. In contrast, humic acid-iron-manganese colloids (HA-Fe/Mn), due to their higher carboxyl content, actually enhance colloid mobility (Zheng et al., 2024). This finding is highly consistent with the conclusion of the present study that humic acid-bound antimony increases the risk of crop uptake. *A. johnsonii* and *R. mucilaginosa* inoculation significantly increased the proportion of iron-manganese oxide-bound and residual Sb and reduced the proportion of the humic acid-bound Sb. Consequently, through their combined effects, *A. johnsonii* and *R. mucilaginosa* inoculation effectively inhibited the absorption and accumulation of Sb by pak choi.

As common resident bacterial taxa in soil, *Pseudomonadota* and *Bacteroidota* play important roles in modulating heavy metal bioavailability (Li et al., 2022). Inoculation with *A. johnsonii* did not significantly alter the total relative abundance of these two bacterial taxa at either the phylum or genus level. In contrast, *R. mucilaginosa* increased the total relative abundance of these two taxa. As key fungal taxa in the environment, *Ascomycota*, *Mortierella*, and *Pichia* play crucial roles in heavy metal immobilization, transformation, and bioremediation through their respective unique mechanisms. As a vast taxonomic group, *Ascomycota* exhibits remarkable biosorption capabilities via hyphae or yeast cells surfaces, where components such as chitin and chitosan passively bind metal ions. Furthermore, *Pichia* species can biotransform heavy metals through redox reactions and methylation, altering their mobility and toxicity (Martorell et al., 2012). *Mortierella*, a plant growth-promoting fungus, often forms mycorrhizal or endophytic associations with plants, enhancing phosphate

nutrition and plant growth, while also exhibiting significant tolerance to multiple heavy metals (such as Cd, Pb, Zn, Cu, and As) (Cui et al., 2017). *A. johnsonii* did not significantly influence the fungal community structure at phylum or genus level, but *R. mucilaginosa* significantly increased the relative abundance of *Mortierella* and *Pichia* in the rhizosphere soil and successfully established itself in the Sb-contaminated environment.

PCoA and RDA revealed that *R. mucilaginosa* inoculation significantly drove variations in bacterial and fungal communities and showed a stronger association between the community structure and environmental factors. Although *A. johnsonii* and *R. mucilaginosa* did not show obvious difference in the variation of Sb speciation in rhizosphere soil, *R. mucilaginosa* inoculation more effectively alleviated Sb accumulation in pak choi. Therefore, it is believed that the regulation of Sb accumulation in pak choi by *R. mucilaginosa* contributes to its effect on Sb speciation and the beneficial microbial community in the rhizosphere soil, while *A. johnsonii* mainly contributes to the regulation of Sb speciation alone.

Conclusions

This study demonstrates that *A. johnsonii* and *R. mucilaginosa* can significantly enhance the pak choi growth and reduce Sb accumulation in a contaminated soil–crop system through their plant growth-promoting traits, such as nitrogen fixation and phosphorus solubilization, which increase soil organic matter and available nitrogen. Critically, both strains promoted the conversion of bioavailable Sb into more stable residual and iron–manganese oxide-bound forms, thereby reducing Sb mobility and plant uptake. *R. mucilaginosa* outperformed *A. johnsonii* in enhancing plant growth and immobilizing Sb, and uniquely reshaped the rhizosphere microbial community by enriching beneficial fungi such as *Mortierella* and *Pichia*, thereby strengthening plant resilience under Sb stress. In summary, this research elucidates a synergistic mechanism of “nutrient activation–speciation stabilization–microbial regulation” underlying microbial remediation of Sb-contaminated soil, providing a theoretical and practical basis for the application of functional microorganisms in sustainable agricultural remediation. Based on the pot experiment results obtained in this study, we plan to continue this research by conducting field trials to verify the practical remediation effects of the two strains and to further dissect the mechanism by which *R. mucilaginosa* reshapes the rhizosphere fungal community. The findings can be developed into microbial remediation agents for antimony-contaminated farmland, ensuring the safety of agricultural products by reducing antimony accumulation in pak choi, with low-cost and environmentally friendly application prospects.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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