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Variety-dependent seed endophytic bacteria enhance stress tolerance to and bioaccumulation of ciprofloxacin in choy sum (*Brassica parachinensis*)

Yi-Ze Wang[1,2†], Hai-Ming Zhao[†], Xian-Pei Huang[1], Yu Zhang[1], Jin-Cheng Ye[1], Nai-Xian Feng[1], Yan-Wen Li[1], Bai-Lin Liu[1], Quan-Ying Cai[1], Lei Xiang[1*], Ce-Hui Mo[1*] and Qing X. Li[3]

Abstract

Background Accumulation of antibiotics in crops threatens human health. However, the mechanisms and effects of microorganisms on the uptake and accumulation of antibiotics in crops remain poorly understood. This study aimed to investigate the impact and underlying mechanisms of seed-borne microbiota in root on ciprofloxacin (CIP) accumulation in two choy sum varieties through amplicon sequencing, multiple statistical analyses, and subsequent validation of key bacteria via isolation and co-culturing with plants.

Results Bacillaceae (mainly *Bacillus*) was enriched specifically in the roots of CIP high-antibiotic-accumulating variety (HAV) via seed-based vertical transmission activated by the root exudate-derived maleic acid. The relative abundance of Bacillaceae was 9.2 to 27.7 times higher in roots of HAV relative to the low-antibiotic-accumulating variety (LAV). The enrichment of Bacillaceae facilitated a cooperative and beneficial bacterial community formed by the deterministic process. The community in HAV could not only stimulate antioxidase activities and decrease membrane lipid peroxidation via secreting indoleacetic acid and siderophore but also promote its biomass, especially the root length and biomass of HAV, thus greatly improving its tolerance to and absorption of CIP. The variety-specific plant-microbial interactions caused 1.6- to 3.2-fold higher CIP accumulation in shoots of HAV relative to LAV shoots.

Conclusions The findings highlight the crucial roles of the seed-borne microbiota in regulating the uptake and accumulation of antibiotics in crops, giving new understanding on the accumulation of organic pollutants in plants, with an emphasis on plant-microbial interactions

Keywords Antibiotic, Ciprofloxacin accumulation, *Brassica parachinensis*, Endophyte, Vertical transmission

†Yi-Ze Wang and Hai-Ming Zhao contributed equally to this work.

*Correspondence:
Lei Xiang
xianglei@jnu.edu.cn
Ce-Hui Mo
tchmo@jnu.edu.cn
Full list of author information is available at the end of the article

Introduction

Antibiotics are widely used in both human and veterinary medicine, with global consumption ranging from 21.1 to 34.8 billion of defined daily doses during the period from 2000 to 2015 [1]. Furthermore, it has been predicted that this level of consumption will double by 2030 [1]. Antibiotics are resistant to metabolism in vivo, with 30–90% of the dosages being released into the environment via manure and sewage [2, 3]. Antibiotic residues raise increasing public concerns [4]. Ciprofloxacin (CIP) is among the most commonly utilized broad-spectrum fluoroquinolone antibiotics. CIP has been extensively detected in agricultural soils, particularly those treated with manure or sewage sludge [5–7]. The CIP levels in soils often surpass the ecotoxic effect trigger value (100 μg/kg) established by the Steering Committee of the Veterinary International Committee on Harmonization [5–7]. CIP in soils is easily absorbed and accumulated in vegetables, potentially threatening the agricultural product safety and human health [8]. Choy sum (*Brassica parachinensis*), one of the most popular *Brassica* vegetables grown in China and sold abroad, is apt to bioaccumulate CIP in the edible parts from the polluted soils [9–11]. Accordingly, there is an urgent need for reducing the CIP accumulation in the vegetables.

Screening and studying low accumulation of varieties (LAVs) of crops against the specific pollutant have become an important strategy to ensure the safety of agricultural products, attracting much attention in recent years [12–14]. Various LAVs against heavy metals (cadmium, lead, mercury, arsenic, nickel, and zinc) have been screened out from different vegetables and grain crops [15–17]. The mechanisms underlying the low accumulation of heavy metals in the LAVs have been gradually revealed at the perspectives of the physiological, biochemical, and molecular levels [18–20]. However, the LAVs against organic pollutants and the underlying mechanisms have been hardly studied [21], especially for the antibiotics. We recently screened out LAV of choy sum against CIP, with up to 2.4- to 3.4-fold differences in shoot CIP concentrations relative to its counterpart high-accumulating variety (HAV), under < 10 mg/kg or 5 mg/L of CIP level [9, 21]. The HAV displayed stronger root CIP uptake, CIP translocation from root to shoot, and shoot resilience to CIP toxicity relative to LAV, thus leading to higher CIP accumulation in its edible shoots [9, 22]. However, the underlying mechanisms that differentiate CIP stress tolerance, as evidenced by significant reductions in biomass, and resultant CIP accumulation in shoots between the two varieties remain elusive.

On the other hand, a large number of microorganisms attach to plant organs or parasitize plants to form the specific plant-associated microbiomes [23–25]. The plant-associated microbiota, especially root microbiota, usually play vital roles in plant growth and health by modulating some critical processes, such as nutrient absorption [26], stress response [27], immune system [28], and disease development [29]. Some studies have also reported key roles of the root microbiota in uptake and accumulation of heavy metals in plants. It is found that the root endophytes can affect the accumulation of heavy metals in plants by changing the host phenotype [23, 30, 31]. For example, the specific root-associated endophytes (Streptomycetaceae, Nocardioidaceae, and Pseudonocardiaceae) of the hyperaccumulator (*Sedum alfredii*) can enhance cadmium/zinc accumulation in shoots by improving biomass and expression of the transporter genes [23, 32]. Arbuscular mycorrhizal fungi can reduce accumulation of cadmium, arsenic, and manganese in crops, by increasing phosphorus uptake and producing insoluble phosphate precipitates [31, 33, 34]. In comparison with the studies on heavy metals, the effects and mechanisms of the plant-associated microbiota on the uptake and accumulation of organic pollutants in plants have been hardly reported [21]. Especially, the effects and mechanisms of the seed-borne microbiota on uptake and accumulation of organic pollutants in plants are yet to be investigated, despite their potential significance in elucidating differential tolerance to pollutant stress and resultant shoot accumulation between the LAVs and HAVs.

The root-associated microbiomes, including both the rhizosphere and endosphere, exhibit inherent differences among various plant species or varieties [23, 35, 36]. Our previous research has revealed significant distinctions in the root-associated microbial communities between the HAV and LAV of choy sum under CIP stress [37]. However, the identification of key differential microbial populations and their role in regulating CIP uptake and accumulation, which subsequently differentiates shoot CIP concentrations between these two varieties, remain unclear. Therefore, it is crucial to identify variety-specific microbial populations and elucidate their impact on shoot CIP accumulation. This knowledge will enhance our understanding of the mechanisms underlying plant-microbial interactions in relation to CIP uptake and accumulation.

In this study, we selected the CIP-LAV of choy sum combined with HAV to investigate (1) their differential seed-borne microbiota in roots under various concentrations of CIP stress (0, 0.2, and 1 mg/L) using profiling of 16S rRNA and internal transcribed spacer (ITS) genes, (2) the effects of seed-borne microbiota on bioaccumulation of CIP in the two choy sum varieties, and (3) the mechanisms underlying varied CIP bioaccumulation

between HAV and LAV from a plant-microbial interaction perspective. Additionally, inoculation experiments were conducted to verify the roles of the key seed-borne microbiota in differentiating shoot CIP bioaccumulation between the two choy sum varieties. The findings from this study are expected to bridge knowledge gaps regarding the effects of seed endophytes on pollutant accumulation in crops and resulting varietal differences between varieties.

Materials and methods

Materials

The LAV (*youlvcutai*) and HAV (*youqingsijiu*) of the CIP for choy sum identified previously by the authors were used in this study [9]. The seeds of the two choy sum varieties were respectively produced at greenhouses of the Vegetable Research Institute of the Guangdong Academy of Agricultural Sciences (Guangzhou, China) under identical soil cultivation conditions. As a result, the plant-associated microbiome demonstrated varietal specificity, including potential horizontal transmission from the environment and possible vertical transmission through the seeds. The plant seeds were surface-sterilized following a meticulously organized sterilization protocol detailed in Supplementary Information T1. Standard CIP (99.0% purity) and its isotopically labeling CIP-d8 (98.7% purity) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China).

Characterization of the seed-borne microbiota in roots

The two used choy sum varieties were respectively used to investigate their seed-borne microbiota in roots in the presence of CIP using an axenic system [36]. In brief, the sterilized Hoagland nutrient solution containing different concentrations of CIP (0.2 and 1 mg/L) was respectively added to the sterilized Magenta boxes. The composition of the used Hoagland nutrient solution per 1.0 L was as follows: 607-mg K_2SO_4, 115-mg $NH_4H_2PO_4$, 493-mg $MgSO_4$, 20-mg EDTA ferric-sodium salt, 15-mg $FeSO_4$, 2.86-mg H_3BO_3, 4.5-mg borax, 2.13-mg $MnSO_4$, 0.05-mg $CuSO_4$, 0.22-mg $ZnSO_4$, 0.02-mg $(NH_4)_2SO_4$, and 945-mg $Ca(NO_3)_2$. The treatment using the same nutrient solution without CIP was set as control treatment. The Magenta boxes with a 1180-mL volume used in each treatment were added with 50 g of sterilized inert substrate (quartz sand). Ten sterilized seeds of choy sum were placed into the Magenta box. Each treatment was conducted in triplicate, with a total of 18 Magenta boxes. The inert substrate used in the Magenta box was designed to fix the seedlings and proved to be negligible for CIP sorption [38]. All the Magenta boxes were placed randomly inside an artificial climate box with temperature = 25 ± 0.5 °C, relative humidity = 60 ± 2%, and 14-h light period [39]. The method for evaluating sterility of the used axenic system is provided in Supplementary Information T2. After 7 days of cultivation, half of the seedlings were collected to obtain their roots under sterile conditions in a SW-CJ-2FD laminar flow cabinet (AIRTECH, Suzhou, China). The obtained roots were immediately frozen in liquid nitrogen and stored at − 80 °C for determining the seed-borne microbiota by 16S rRNA and ITS genes amplicon sequencing in Illumina platform. More details regarding the sequencing bioinformatics analysis of sequencing data are listed in the Supplementary Information T3. The other half of the collected roots were used to measure biomass and CIP concentrations. Meanwhile, the CIP concentration in the used Hoagland nutrient solution was determined for evaluating the concentration factor of CIP in the choy sum varieties. The methods for extraction and analysis of CIP and the statistical analysis for the obtained data were available in Supplementary Information T4, T5, and Table S1.

Validation experiment based on plant-bacterium co-culturing

A seed-borne isolate of core *Bacillus* strain (hereafter referred to BpB13), isolated from HAV choy sum roots using the dilution plating technique (Supplementary Information T6) [40], was used in a plant-bacterium co-culture experiment to assess its effects on CIP accumulation in both LAV and HAV. The 16S rRNA gene sequence of BpB13 (Supplementary Information T7) was aligned with the sequences of dominant *Bacillus* OTUs (Supplementary Information T8) using BLASTn, thereby confirming its predominant role within the HAV microbiome. The antibiogram of the isolated *Bacillus* strain against CIP and the corresponding minimum inhibitory concentration (MIC) were determined using a twofold serial microdilution method [41], with the details provided in Supplementary Information T9. For the plant-bacterium co-culture experiment, four sterilized seeds of each choy sum variety were placed into the sterilized Magenta box loaded with 1/2 MS solid medium (containing 3% sucrose and 1% agar) for each treatment [42]. Such a co-culture included four treatments for each variety, i.e., (1) pure medium (control), (2) the medium containing CIP (labelled as CIP), (3) the medium containing no CIP and inoculated with BpB13 (labelled as B), and (4) the medium containing CIP and inoculated with BpB13 (labelled as CIP + B). CIP concentration and inoculation dosage of the *Bacillus* strain in the co-culture were set as 0.2 mg/L and ~ 1×10^6 cells per milliliter medium, respectively. Each treatment was conducted in triplicate, with a total of 24 boxes for the LAV and the HAV. After 14 days of cultivation, root length, plant biomass, CIP

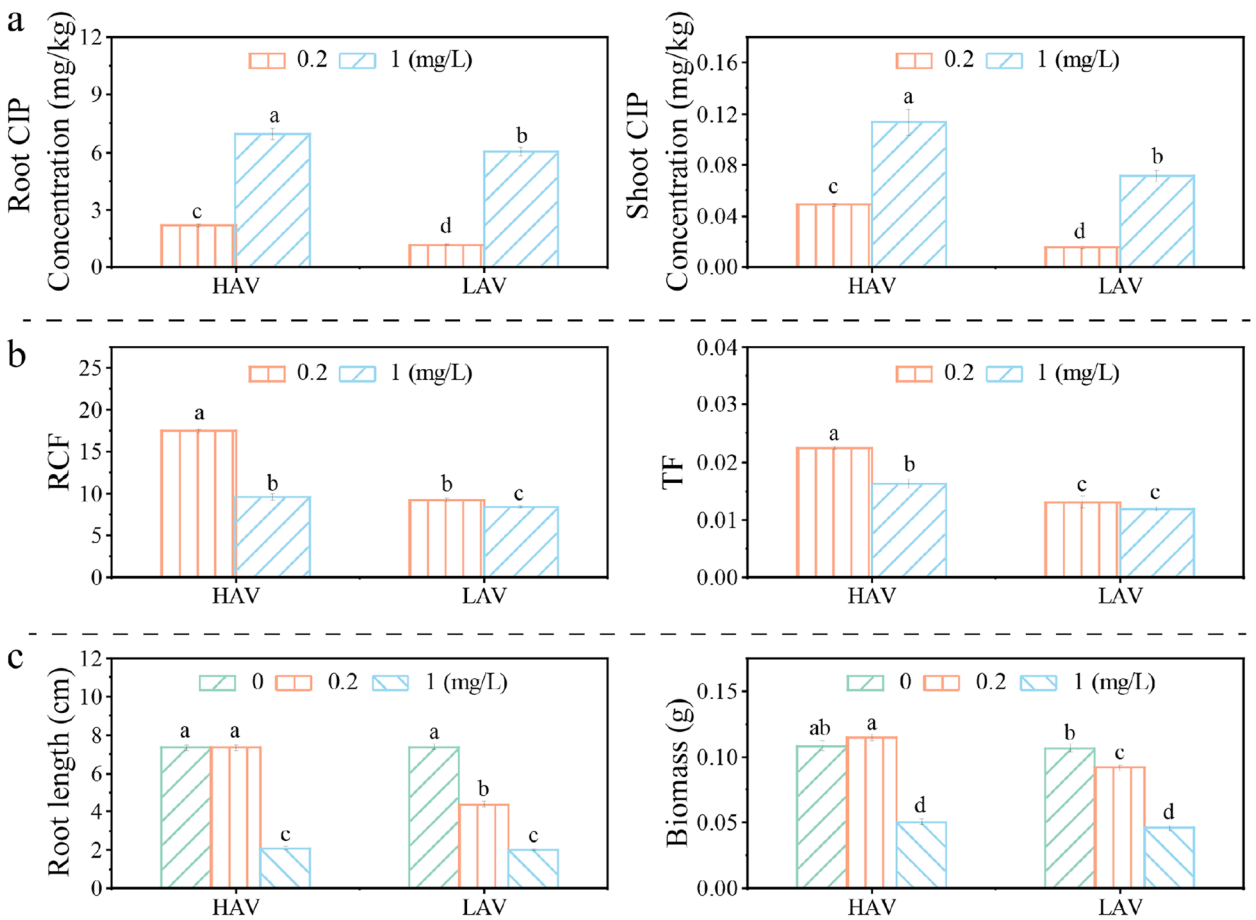

Fig. 1 CIP concentration (**a**), CIP root concentration factor (RCF) and translocation factor (TF) (**b**), and biomass (**c**) of the two choy sum varieties (LAV and HAV) upon exposure to different levels of CIP (0, 0.2, and 1 mg/L). The various lowercase letters indicate significant differences among the different CIP groups for each variety at $p < 0.05$

concentrations, antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)], and malondialdehyde (MDA) contents in plants were measured. Meanwhile, a portion of seedling root samples was surface-sterilized to ensure that only the endophytic population was analyzed. After sterilization, the sample was flash-frozen in liquid nitrogen and stored at − 80 °C. The abundance of endophytic *Bacillus* in the seedling roots was determined using 16S rRNA gene amplicon sequencing. In addition, the plant growth-promoting (PGP) properties of the BpB13 were determined, including secretion of indoleacetic acid (IAA), activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, production of siderophore, and solubilization of phosphate. More details on root surface sterilization, 16S rRNA gene sequencing and analysis, the characterization of relevant enzyme activities in plants, and the determination of PGP properties of the *Bacillus* strains are provided in the Supplementary Information T1, T3, T10, and T11, respectively. To elucidate the impact of IAA on CIP bioaccumulation in the two choy sum varieties, an experiment involving the addition of IAA (100 μM) was carried out under varying CIP concentrations (0, 0.2, and 1 mg/L) and the aforementioned cultivation conditions, excluding BpB13 inoculation. In addition, a chemotaxis assay was conducted to evaluate the chemotactic response of BpB13 towards the primary components of low-molecular-weight organic acids (LMWOAs) derived from both tested choy sum varieties. More details are provided in Supplementary Information T12.

Results

Variety-dependent CIP accumulation

The effectiveness of the surface-sterilized seeds and the axenic system was validated by the results presented in Figs. S1 and S2. CIP concentrations in both varieties significantly increased with the increasing CIP concentration in the nutrient solutions of the axenic system ($p < 0.05$) (Figs. 1a, S3). CIP concentrations in the HAV were detected as 2.2 and 7.0 mg/kg (dw) in roots and

0.05 and 0.1 mg/kg (dw) in shoots in 0.2 and 1 mg/L of CIP treatments, respectively (Fig. 1a), which were significantly higher than those of the LAV, differentiating 1.2- to 1.9-fold in roots and 1.6- to 3.2-fold in shoots ($p < 0.05$). Correspondingly, both RCF values (9.6–17.5) and TF values (0.016–0.022) of the HAV were 1.1- to 1.9-fold higher than those of the LAV (Fig. 1b). These results confirmed the stable variety-dependent CIP accumulation in choy sum varieties, as reported previously by the authors [9]. On the other hand, the HAV had higher biomass (1.01- to 1.25-folds) and root length (1.01- to 1.67-folds) than LAV, with significant differences observed in 0.2 mg/L of CIP treatment (Fig. 1c). This result indicated that the HAV had higher tolerance to CIP, as reported previously [9]. However, the differences in both biomass and root length between the HAV and the LAV in high CIP treatment (1 mg/L) were insignificant, because both of them suffered from severe inhibition on the growth, which could weaken the biomass differences [9].

Variety-dependent and seed-borne microbiota under the CIP stress

A total of 998,065 bacterial and 503,440 fungal high-quality clean reads regarding HAV and the LAV under CIP stress were yielded, which were identified to 2716 bacterial and 823 fungal OTUs across all samples, respectively. Most of the bacterial OTUs (99.8%) could be assigned to three dominant phyla, including Proteobacteria (2.2–54.3% relative abundance, hereafter), Firmicutes (45.5–97.8%), and Actinobacteria (0.04–1.6%) in both varieties (Fig. S4a). In contrast, 82.8% of the fungal OTUs remained unidentified. Only 15.1% of the OTUs were classified into Ascomycota (with a range of 0.6 to 22.0%) and Basidiomycota (with a range of 0.3 to 0.4%) across both varieties. Additionally, 2.1% of the OTUs were assigned to other phyla, collectively contributing less than 0.1% relative abundance (RA) in both varieties (Fig. S4b).

To assess the compositional differences in seed-borne microbiota between the LAV and HAV, the β-diversity was investigated. The results revealed distinct clustering of bacterial communities associated with the HAV and the LAV along the first coordinate ($p < 0.05$) (Fig. 2a). Furthermore, both varieties in each CIP treatment also had different clusters of the bacterial communities ($p < 0.05$, Table S2). However, the fungal community composition with an obvious separation was observed just in the LAV under 1 mg/L of CIP treatment (Fig. S5). The PERMANOVA results further suggested the composition of seed-borne bacterial communities was jointly affected by both varieties (22.6%; $p < 0.01$) and CIP concentrations (22.3%; $p < 0.05$), but the fungal community composition was mainly affected almost only by the CIP concentrations ($p < 0.05$, Table S2). These results indicated that only the composition of seed-borne bacterial communities was variety dependent.

Alpha-diversity analysis indicated obvious differences in both Chao1 and Shannon indices between the two varieties, with significant differences in 0 and 0.2 mg/L of CIP treatments (Fig. 2a, $p < 0.05$). Taxonomic analyses of bacterial communities indicated significant differences ($p < 0.05$) at the family level between the HAV and the LAV under all the CIP treatments (Fig. 2b). Specifically, the RA of Bacillaceae, including its main member *Bacillus*, was significantly higher in the seeds and roots of HAV compared to LAV ($p < 0.01$). In contrast, the RAs of Lachnospiraceae and Enterobacteriaceae were significantly lower in roots of HAV compared to those of the LAV ($p < 0.01$, Fig. 2c).

A null model was employed to evaluate the roles of deterministic and stochastic processes in bacterial community assembly, finding that the mean modified stochasticity ratio (MST) value of LAV exceeded 0.5 in the control group but declined below 0.5 under CIP treatments at concentrations of 0.2 and 1 mg/L, indicating a shift from stochastic to deterministic processes (Fig. S6). In contrast, HAV exhibited MST values less than 0.5 across all treatments, suggesting a dominant role for deterministic processes in bacterial community assembly regardless of CIP stress.

The co-occurrence networks of seed-borne bacterial communities in the HAV and LAV under different CIP stress were constructed based on Spearman's correlations at the OTU level, with $RAs > 0.01\%$ (Fig. 3a). Compared to HAV, the networks in LAV exhibited higher complexity and connectivity under all treatments, with 1.33–1.45 times more nodes, 3.87–4.78 times more edges, and 2.90–3.50 times higher degrees (Fig. 3b, Table S3). Conversely, HAV displayed greater modularity (0.49–0.609) and positive correlations (75.3–81.5%), compared to LAV which had lower modularity (0.458–0.535) and positive correlations ranging from 58.4 to 64.6% across all treatments (Fig. 3b, Table S3). These results suggested that the seed-borne bacterial community was characterized by increased complexity and connectivity in LAV while being more specific and cooperative in HAV. CIP-bacteria co-occurrence networks were constructed using the top 500 OTUs to investigate the correlation between CIP concentration and seed-borne bacterial communities in HAV and LAV. The analysis revealed that CIP had a significantly greater impact on the bacterial community in LAV, affecting 229 OTUs (45.8% of the total), compared to only 46 OTUs (9.2%) in HAV (Fig. S7). Specifically, the 229 OTUs negatively influenced by CIP in LAV encompassed a broader taxonomic range, including

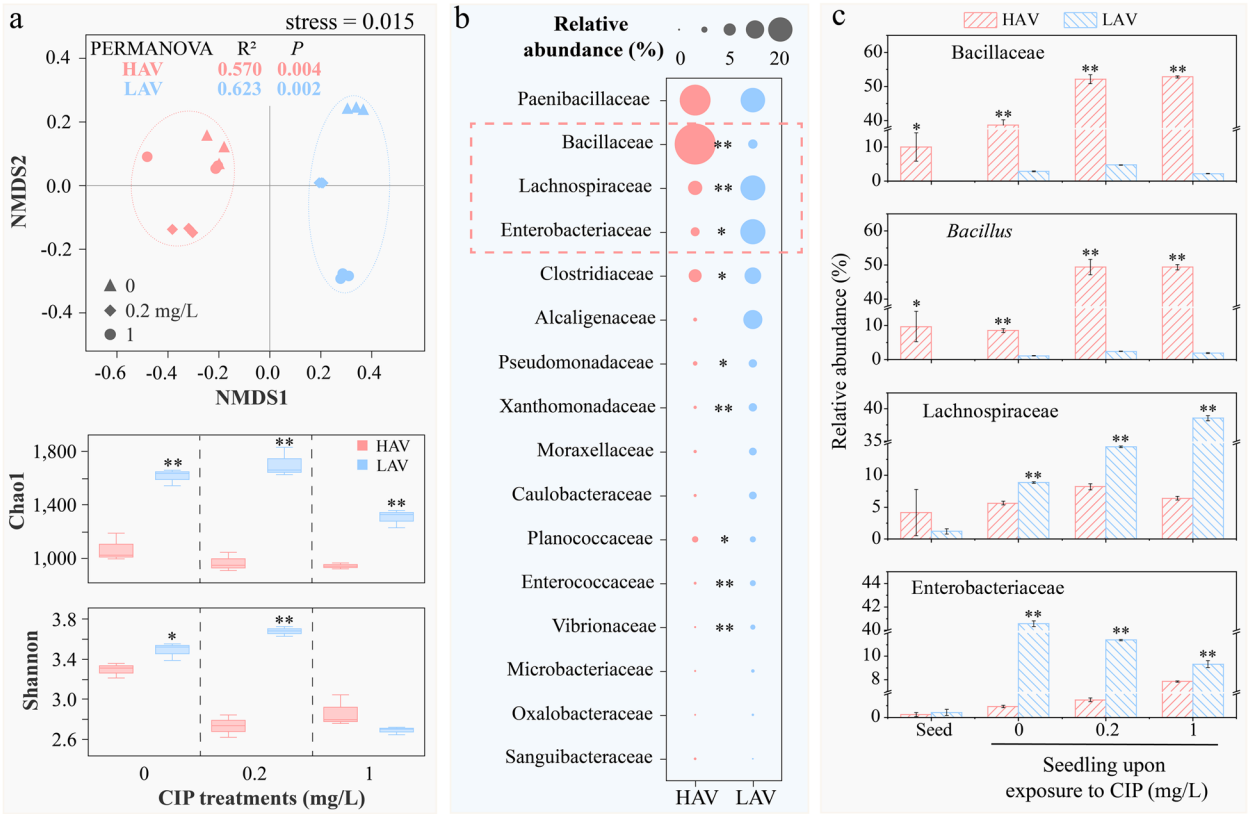

Fig. 2 Characterization of seed-borne bacterial communities of the HAV and the LAV exposed to different levels of CIP (0, 0.2, and 1 mg/L) under axenic environment: **a** nonmetric multidimensional scaling (NMDS) ordinations and PERMANOVA based on Bray–Curtis dissimilarity and alpha diversity based on Chao1 and Shannon indices, **b** the relative abundances of dominant families between LAV and HAV across all three concentrations of CIP, and **c** comparisons in the differential key bacteria between LAV and HAV at each CIP concentration. "*" and "**" represent significant differences between the HAV and the LAV at $p < 0.05$ and $p < 0.01$, respectively

Paenibacillaceae (50 OTUs), Enterobacteriaceae (46 OTUs), Moraxellaceae (22 OTUs), Clostridiaceae (20 OTUs), Lachnospiraceae (18 OTUs), Pseudomonadaceae (13 OTUs), Bacillaceae (11 OTUs), and Xanthomonadaceae (9 OTUs). In contrast, the 46 OTUs affected by CIP in HAV belonged to Paenibacillaceae (27 OTUs), Bacillaceae (11 OTUs), Clostridiaceae (3 OTUs), and Enterobacteriaceae (2 OTUs).

Effects of variety-specific and seed-borne bacteria on CIP bioaccumulation in choysum varieties

Beta-diversity analysis revealed a distinct separation of the seed-borne bacterial communities between the two varieties under CIP stress (Fig. 2a). Notably, despite this separation, HAV and LAV still shared specific core bacteria in their seeds in response to CIP exposure. These core genera, including Unassigned Enterobacteriaceae, Unassigned Lachnospiraceae, *Clostridium*, *Paenibacillus*, and *Bacillus*, were consistently detected across all replicates with a prevalence of 100% and a detection threshold RA value exceeding 0.50% (Fig. 4a). The Kruskal–Wallis test suggested the variety preference of the core genus to the HAV and the LAV, respectively (Fig. 4b). Significantly higher RAs (23.0- to 27.7-fold) of core *Bacillus* were observed in the HAV than in the LAV ($p < 0.05$), while significantly higher RAs of core Unassigned Enterobacteriaceae (1.3- to 7.8-fold) and core Unassigned Lachnospiraceae (1.7- to 6.5-fold) were found in the LAV relative to the HAV in the two CIP treatments ($p < 0.05$). Correlation analysis among these core taxa revealed a significantly positive relationship between *Bacillus* and Unassigned Enterobacteriaceae in HAV, whereas a negative correlation was observed between Unassigned Enterobacteriaceae and Unassigned Lachnospiraceae in LAV (Fig. S8). The differences in the core bacteria between the HAV and the LAV could contribute to the CIP bioaccumulation variation.

Additionally, correlation analyses also indicated significant positive correlations between shoot CIP concentrations (ShootCIP), RCFs, and TFs with the RAs of core *Bacillus* ($p < 0.05$, Fig. 4b). However, significantly negative correlations were found for both plant biomass

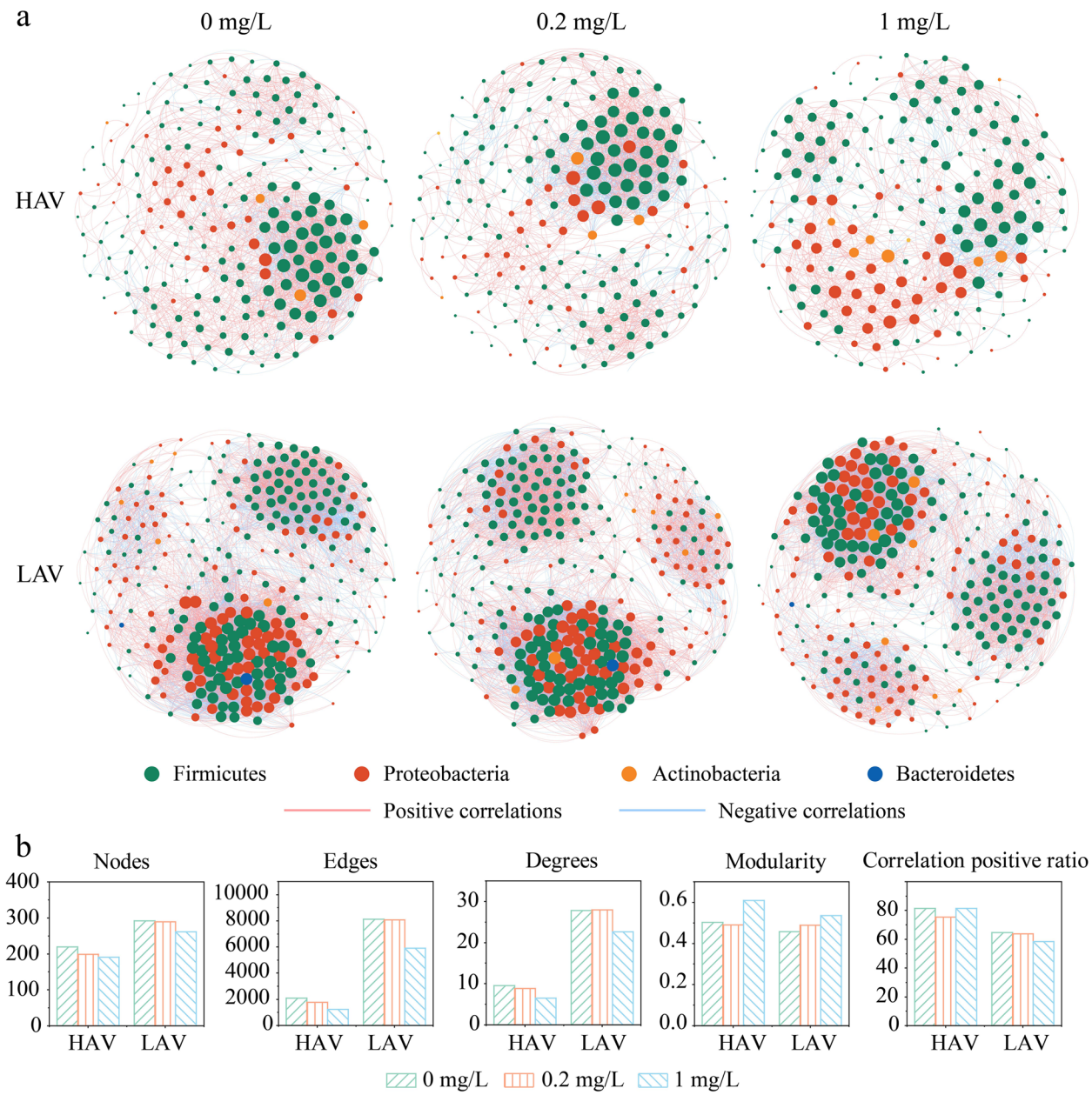

Fig. 3 Co-occurrence network analyses (**a**) and topological characterizations (**b**) of the seed-borne bacterial communities of HAV and LAV exposed to different levels of CIP (0, 0.2, and 1 mg/L) grown in an axenic environment. Connection stands are set as Spearman's correlation >|0.6| and statistically significant at $p < 0.05$. The color of the connections represents the different correlation types (positive and negative). Nodes represent OTUs, and the size of each node is proportional to the number of connections (degree). OTUs are colored by taxonomy

and root length with the RAs of Unassigned Lachnospiraceae, as well as both RCFs and TFs with the RAs of Unassigned Enterobacteriaceae. These results suggested that high enrichment of the core *Bacillus* could be conducive to improving CIP bioaccumulation in the plant. On the contrary, Unassigned Lachnospiraceae might hinder the biomass, especially root length of the two varieties, and the resultant CIP uptake and translocation in the choy sum roots.

A structural equation model (SEM) was further developed to assess the direct and indirect effects of multifactors concerning the seed-borne core bacteria on CIP bioaccumulation in the two choy sum varieties. The developed SEM displayed satisfactory fitness, with $\chi^2/df < 1.082$, $P = 0.364$, $CFI = 0.971$, and

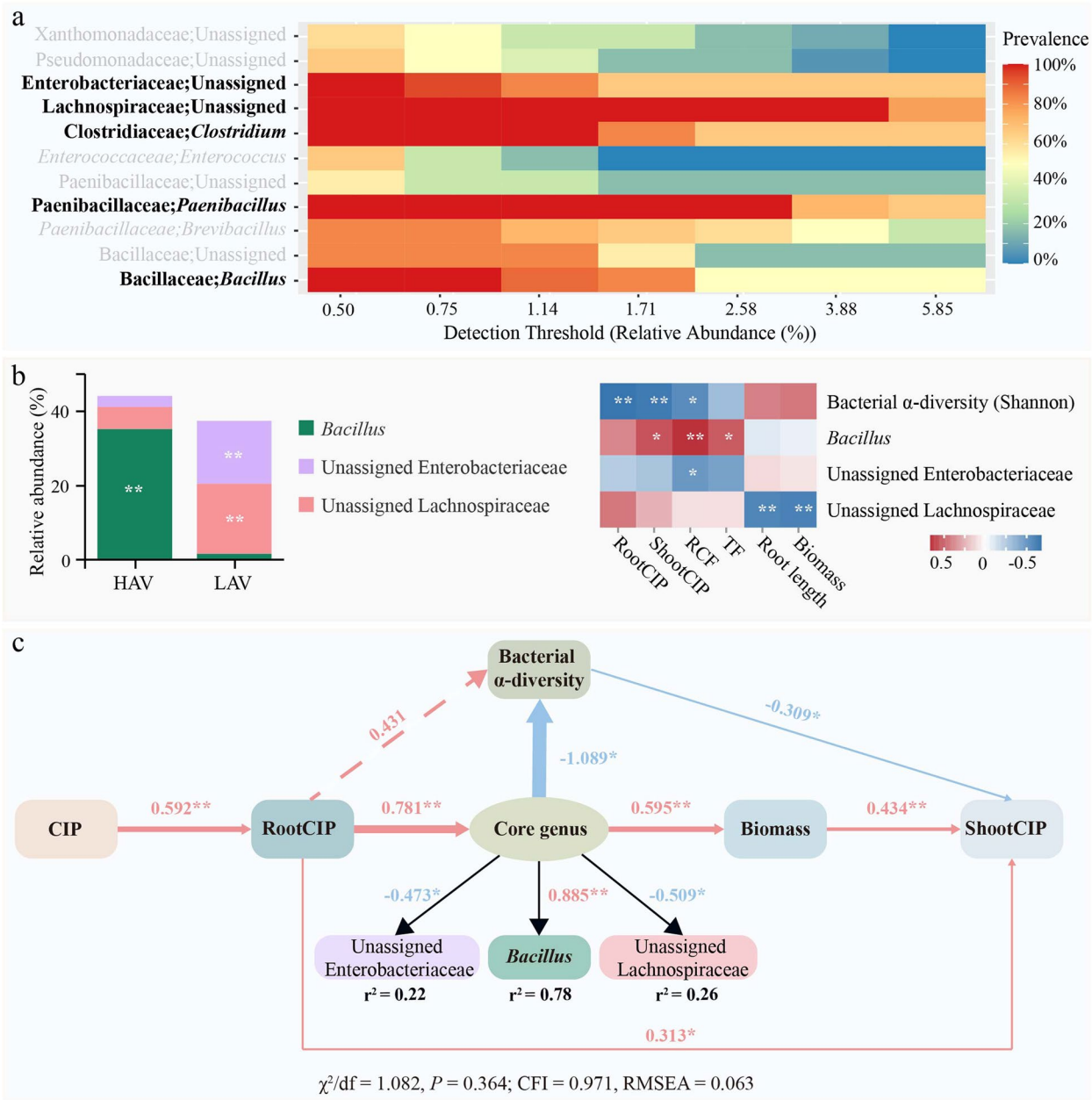

Fig. 4 Core bacteria at the genus level in roots of both choy sum varieties (**a**), comparisons in differential core bacteria between the HAV and the LAV, and correlations between the relative abundances of differential core bacteria and phenotypic parameters of the HAV and the LAV (**b**), and the direct and indirect influences of the differential core bacteria on CIP bioaccumulation using structural equation model (SEM) (**c**). In the subfigure (**c**) regarding the correlations, pink and blue arrows indicate positive and negative relationships, respectively; dotted arrows represent nonsignificant paths ($p > 0.05$); numbers adjacent to arrows are standardized path coefficients. "*" and "**" represent significant level of multifactors at $p < 0.05$ and $p < 0.01$, respectively. RootCIP, root CIP concentration; ShootCIP, shoot CIP concentration

RMSEA = 0.063 (Fig. 4c). It found that root CIP concentrations were positively related to the CIP treatment concentrations and greatly affected the shoot CIP concentrations by the direct and indirect pathways (Fig. 4c). In the direct pathway, the root CIP concentrations had significantly positive effects on the shoot CIP concentrations ($p < 0.05$). As for the indirect pathway, root CIP concentrations also had significantly positive contributions to the shoot CIP concentrations ($p < 0.05$). Such a contribution was essentially linked to several positive correlations, including root CIP concentrations vs core bacteria abundances, the core genus

Bacillus abundance vs the variety biomass, and the variety biomass vs shoot CIP concentrations. The indirect pathway made an important contribution to shoot CIP concentrations, based on the 1.39- to 2.50-fold higher standardized path coefficients compared to the direct pathway. Notably, the positive contribution of the core bacteria to shoot CIP concentrations was primarily attributed to the enrichment of the core genus *Bacillus* in the HAV, rather than the other two core genera (Unassigned Lachnospiraceae and Unassigned Enterobacteriaceae) enriched in the LAV. The core genus *Bacillus* exhibited a significantly positive correlation with biomass and shoot CIP concentration, displaying the highest standardized path coefficient (0.885) among the three core bacteria (Fig. 4b, c). This predominant contribution of the core genus *Bacillus* could be associated with the largest RA differences (23.0- to 27.7-folds) between the two varieties under CIP exposure. Furthermore, the core bacteria abundances showed standardized negative effects on bacterial α-diversity. These results suggested that variety-specific enrichment of core bacteria, especially *Bacillus* in the HAV, may lead to a reduction in bacterial α-diversity. Such specific enrichment of *Bacillus* could potentially promote increased biomass and CIP accumulation in shoots of the HAV.

Inoculation of seed-borne *Bacillus* strain BpB13 enhanced CIP accumulation in choy sum varieties

To further clarify the effects of the seed-borne core bacteria on shoot CIP accumulation, root homogenates from both HAV and LAV exposed to CIP (0.2 mg/L) under axenic condition were used to isolate the target strains. This concentration of CIP was selected because it not only promoted *Bacillus* enrichment in both HAV and LAV roots (Fig. 2) but also exhibited relatively lower toxicity to their biomass, particularly for HAV (Fig. 5). A core *Bacillus* strain, labelled as BpB13, which was enriched in HAV, was successfully isolated (Figs. S9 and S10). BLAST analysis revealed that the 16S rRNA gene sequence of BpB13 exhibited 100% identity with that of the dominant *Bacillus* OTU2 (Table S4). Furthermore, BpB13 also showed the relatively high sequence similarity (87.01 to 98.37%) with other *Bacillus* OTUs. These results substantiated that BpB13 acted as a predominant member (i.e., core *Bacillus* strain) within the HAV microbiome (43.02% ± 2.53%, Fig. S11 and Table S4). The MIC of BpB13 against CIP was determined as 0.25 mg/L (Fig. S12). On the other hand, no seed-borne core bacteria associated with Lachnospiraceae and Enterobacteriaceae were isolated from LAV.

Correlation analysis and SEM showed the positive effects of the *Bacillus* on shoot CIP accumulation and plant biomass, respectively (Fig. 4). Accordingly, BpB13 was inoculated into both HAV and LAV in sterilized Magenta box for plant-bacterium co-culture experiments to investigate its impact on CIP accumulation in these choy sum varieties (Figs. 5 and 6). After inoculating BpB13, the *Bacillus* abundance was generally much higher (3.2-fold) in the HAV than in the LAV under the CIP stress (Fig. 6c). A chemotaxis assay demonstrated that the higher level of malic acid secretion by HAV could be responsible for its higher enrichment of BpB13 (Fig. S13).

BpB13 significantly increased the biomass, root length, root/shoot CIP concentration (Fig. 5), and antioxidant enzyme activities (e.g., SOD, POD, and CAT) of both varieties while reducing their MDA contents induced by CIP stress (Fig. 6a, b). In this study, BpB13 was identified as a siderophore producer (Table S5), which enhanced plant stress resistance [43]. Moreover, this bacterium also secreted a remarkably high concentration of the phytohormone IAA (82.2 μg/mL, Table S5), which was conducive to plant growth. The IAA concentration produced by BpB13 was 2.6 to 34.8 times higher than that reported for other *Bacillus* strains in previous studies [23, 44, 45]. This increased IAA production may enhance the host plant capacity to capture organic pollutants. Indeed, we found that the addition of IAA (100 μg/L) significantly increased CIP concentrations in both varieties, by 1.3–2.3 times in roots and 3.5–8.3 times in shoots, respectively (Fig. S14). Accordingly, the isolated seed-borne strain (BpB13) enriched in HAV roots could promote CIP bioaccumulation in both choy sum varieties by improving their biomass and stress resistance.

Discussion

Variety-dependent transmission of seed-borne bacteria under CIP stress

We conducted a relatively ideal axenic cultivation system to investigate the impact of seed-borne bacteria on the antibiotic accumulation in plant. Although the most ideal condition for assessing bacterial-plant interactions would be to develop a seed model completely free of seed endophytes, this is currently challenging. This is primarily due to the fact that choy sum (*Brassica*) possesses non-endospermic seeds with cotyledons functioning as the exclusive food storage organs [46]. Removing the cotyledons could probably eliminate most of the seed endophytes but also inevitably impair seed germination and seedling survival of the choy sum [46, 47]. Despite the challenge, the development of an ideal axenic cultivation system with a germ-free seed model remains a significant area for future research, which is helpful to further understand the effects of the bacterial-plant interactions on the antibiotic accumulation in plant.

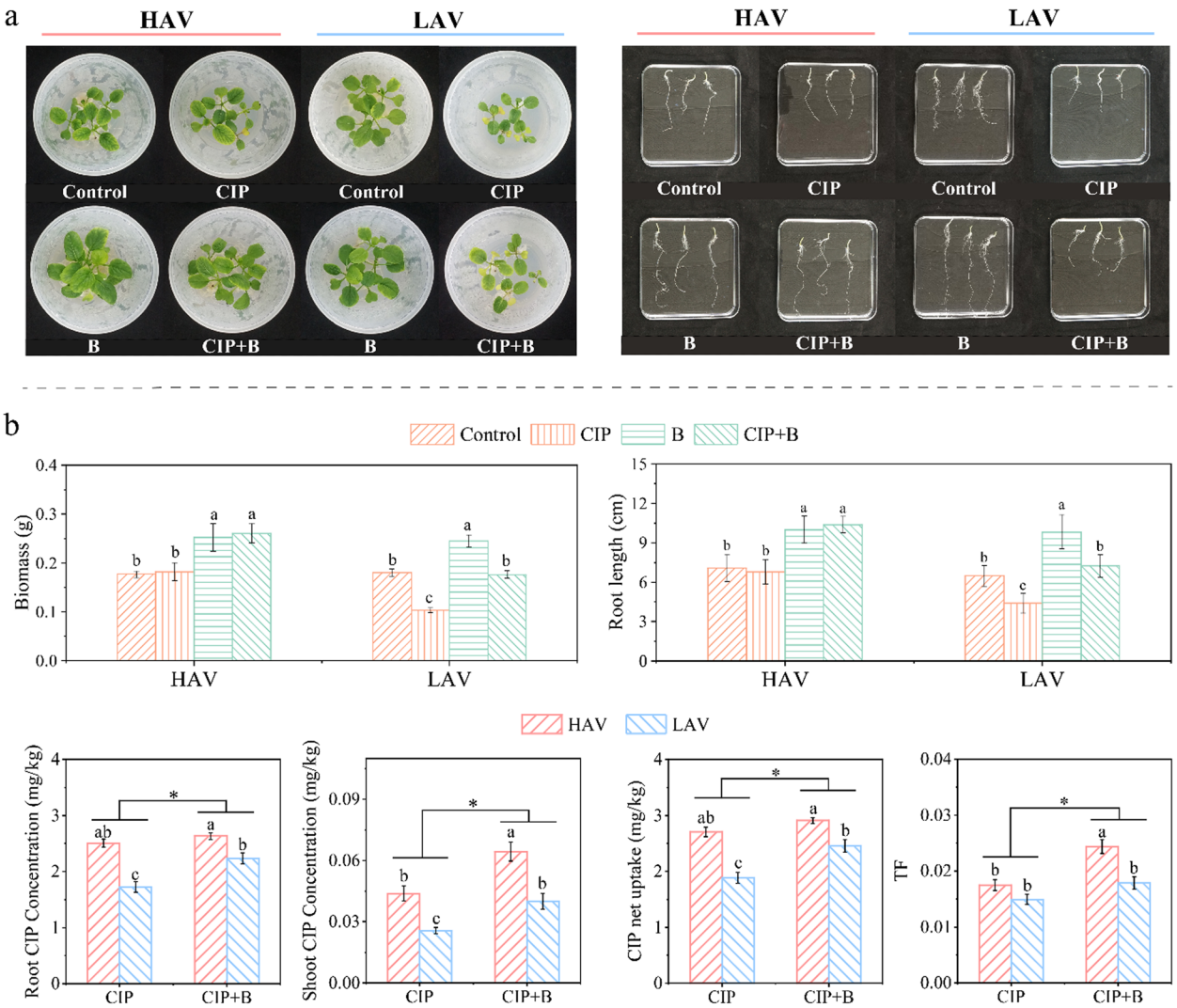

Fig. 5 Pictures of the seedlings and roots of the HAV and the LAV cultivated in the axenic environment inoculated with *Bacillus* strain BpB13 (**a**) and promotion effects of *Bacillus* strain BpB13 inoculation on biomass and CIP bioaccumulation (**b**). The various lowercase letters indicate significant differences among different groups at $p < 0.05$ level according to one-way ANOVA. "*" represents the differences between BpB13 inoculation groups (B, CIP + B), and without BpB13 inoculation groups (control, CIP) are significant at $p < 0.05$, according to paired samples *t*-test, respectively. The CIP concentration was set as 0.2 mg/L

The endophytic microbiome can be assembled by two modes, i.e., (1) horizontal transmission from the environment to the host and (2) vertical transmission from parent to offspring, especially seed-based transmission [23, 48]. Although horizontal transmission is considered as the main approach for community assembly of root endophytes, the seed-based vertical transmission of endophytes has been commonly found in many plant species, such as Brassicaceae, rice, *Phaseolus vulgaris*, and grass, where the seed endophytes can be transmitted to newly developed rhizosphere compartments or root endosphere [48–50]. It is worth noting that the associations between the vertical/horizontal transmission of seed endophytes and the plant tolerance to organic pollutants and the resulting pollutant accumulation remain elusive.

Choy sum is a representative Brassicaceae plant [11]. This study performed the plant cultivation in a strictly axenic condition, ensuring that the core bacteria in roots of the HAV and the LAV were derived from the seed endophytes via vertical transmission [48, 51]. Moreover, remarkable differences were observed in the core bacteria of the both varieties, including *Bacillus*, Unassigned Enterobacteriaceae, and Unassigned Lachnospiraceae (Figs. 2c and 4b). Among the seed microbiota, *Bacillus* showed a higher abundance (~ 10%), with 1000-fold

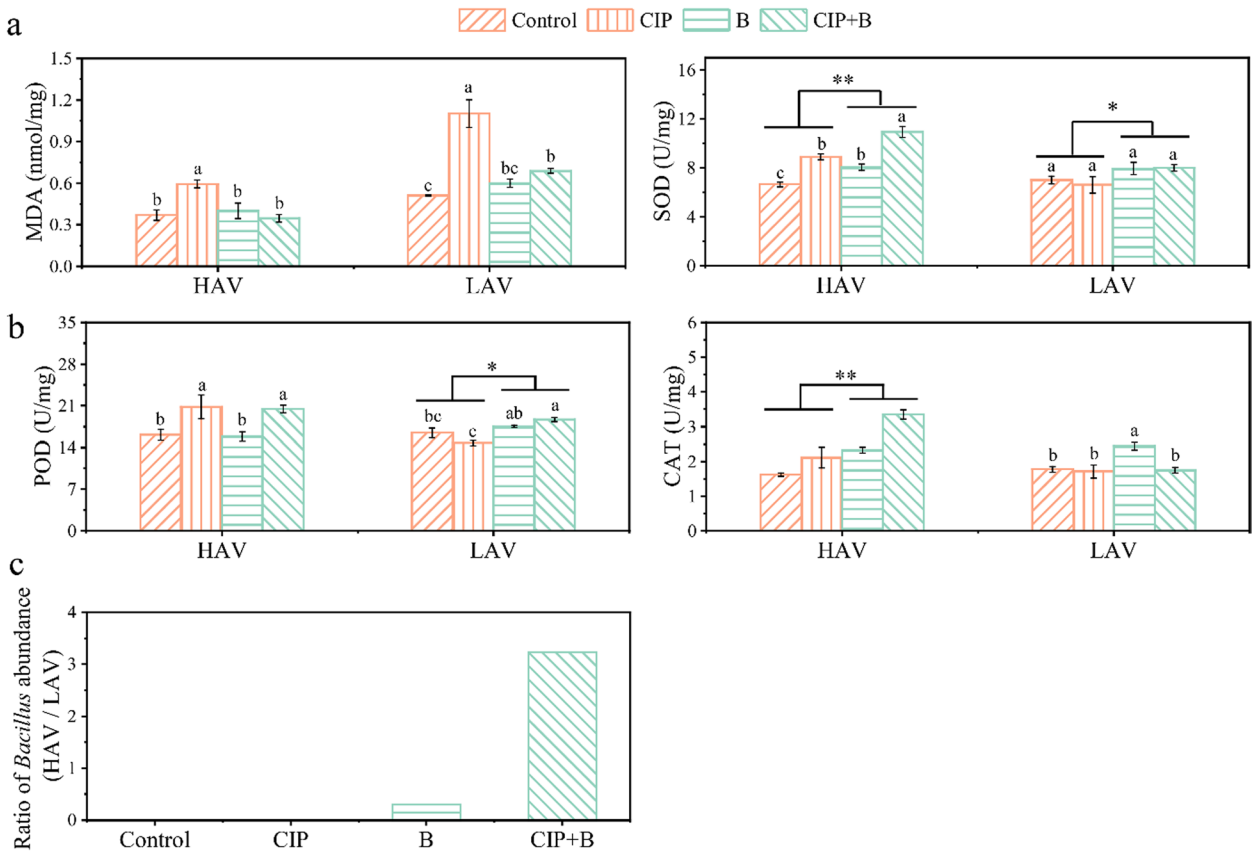

Fig. 6 Effects of *Bacillus* strain BpB13 inoculation on antioxidase activities and malondialdehyde (MDA) contents of the HAV and the LAV under CIP exposure (**a**, **b**). Ratio of *Bacillus* abundance in the seedling between HAV and LAV inoculated with the seed-borne strain BpB13 (**c**). The various lowercase letters indicate significant differences among different groups at $p < 0.05$ level according to one-way ANOVA. "*" represents the differences between the BpB13 inoculation groups (B, CIP + B) and the non-BpB13 inoculation groups (control, CIP), and HAV and LAV were significant at $p < 0.05$, according to paired sample *t*-tests and Mann–Whitney *U*-test. The CIP concentration was set as 0.2 mg/L

higher abundance in the HAV than the LAV (~0.01%) (Fig. 2c). The RA difference of *Bacillus* between the HAV and the LAV seedlings was reduced in the control treatment (7.8-fold) but increased in the CIP treatments (20.4- to 26.2-fold), since the *Bacillus* abundance increased sharply in the HAV seedlings (to ~50%) but slowly in LAV seedlings (to ~2%) under CIP exposure (Fig. 2c). Vertical transmission of seed endophytes commonly happens in the early stages of plant development, e.g., seed germination and seedling development [48, 51, 52]. Such a transmission process usually needs a period of adjudication for the transmitted seed endophytes to adapt themselves to the new niches and thus survive along with the host development by providing the benefits to the host [51].

Notably, the differences in CIP susceptibility of seed-borne bacterial communities between HAV and LAV also could impacted their microbial communities and the corresponding vertical transmission. The co-occurrence network analysis revealed that the CIP susceptibility of seed-borne bacterial communities is variety dependent. CIP exposure impacted a larger proportion (45.8%) of bacterial members in the LAV, relative to HAV (9.2%) (Fig. S7a and b). Furthermore, substantially more negative correlations were observed in LAV compared to HAV (Fig. S7c), suggesting a more extensive disruption of microbial interactions in LAV upon CIP exposure. Wherein, the dominant *Enterobacteriaceae* OTU1 in LAV exhibited a strong negative correlation with CIP concentration, suggesting that this key taxon was highly sensitive to CIP. In contrast, the dominant *Bacillus* OTU2 in HAV was absent from the negative CIP-bacteria network (Fig. S7b), implying minimal CIP-induced disruption. These results suggest that the seed-borne bacterial community in LAV is more susceptible to CIP than that in HAV, resulting in more pronounced shifts in microbial community composition following CIP exposure. This lower susceptibility of HAV bacterial communities to CIP could facilitate survival and vertical transmission of the dominant *Bacillus*.

Bacillus often exhibits strong PGP functions, such as the production of IAA and siderophores [23]. These PGP functions may facilitate the vertical transmission and survival of *Bacillus* within plant rhizocompartments. However, further studies are needed to elucidate the mechanisms underlying the vertical transmission of *Bacillus* and its relationship with other PGP functions, such as formation of endospores, cell motility, and tolerance to high osmotic pressure [53, 54]. Additionally, the *Bacillus* can enhance plant transpiration rate and upregulate the abundance of aquaporin-associated genes and proteins, thus promoting the host resistance to the biotic and abiotic stresses [55–57]. Accordingly, the *Bacillus* abundance in root microbiota of the two varieties was significantly increased under CIP stress, especially for the HAV, leading to a larger root *Bacillus* abundance difference between the two varieties. These results indicated that there was a higher efficiency for the vertical transmission and survival of the *Bacillus* in the HAV relative to the LAV, which was helpful to the tolerance to CIP exposure and to reducing the stress damage (Fig. 5).

Enterobacteriaceae and Lachnospiraceae were also vertically transmitted from seeds to seedlings in the both choy sum varieties, exhibiting distinct RA variation trends between HAV and LAV compared to *Bacillus*. The abundances of both the bacteria in seeds were similar but varied significantly in seedlings ($p < 0.05$, Fig. 2c). Members of Enterobacteriaceae are frequently reported to perform beneficial functions for promoting plant growth and improving plant tolerance to biotic and abiotic stresses [58, 59]. Its abundance was increased slightly in the HAV seedlings but decreased sharply in the LAV seedlings with the increasing CIP concentration. Even so, the Enterobacteriaceae abundance in seedling was always lower in the HAV than the LAV. This result may be attributed to the higher abundance of *Bacillus* (~ 50%) and the resulting higher PGP effects in the HAV, which improved plant resistance to CIP stress and benefited both the plant and the other seed-borne bacteria. This is supported by the results that there were significantly positive RA correlations between the *Bacillus* and Enterobacteriaceae in the HAV (0.68 of Spearman's correlation, Fig. S8), and the improvement correlations between biomass and stress resistance in both varieties inoculated with *Bacillus* strain (Fig. 5). As for Lachnospiraceae, it often occurs in endophytic bacterial communities of the crop susceptible to diseases, exhibiting bacterial decay symptoms [60, 61]. The RA of Lachnospiraceae in the LAV seedlings increased sharply with the increasing CIP concentration (to ~ 40%, Fig. 2c), which may be associated with the reduced biomass and the increased pernicious bacteria (Lachnospiraceae) due to CIP stress. On the contrary, the RA of Lachnospiraceae in the HAV seedlings kept low abundance (~ 5%) under the CIP stress, likely owing to the *Bacillus*-enhanced resistance to the pernicious bacteria (Lachnospiraceae) against the bacterial decay in HAV seedlings.

Besides vertical transmission, the roots could also recruit *Bacillus* from the environment via potential horizontal transmission, since the RA of *Bacillus* in roots of both varieties inoculated with the *Bacillus* strain was significantly increased, regardless of the CIP stress (Fig. 6c). It is worth noting that *Bacillus* abundance was generally much higher (3.2- to 12.9-fold) in the HAV than in the LAV, especially under the CIP stress (Figs. 2b, c, 6c), suggesting a specific enrichment of *Bacillus* in the HAV. The *Bacillus*-associated PGP functions and the increased CIP tolerance may be potentially responsible for high CIP uptake and accumulation in the HAV, highlighting the microbiological mechanism underlying CIP uptake and accumulation regulated by plant-microbial interactions in the HAV.

Variety-dependent and seed-borne bacterial community assembly affected CIP accumulation in choy sum

This study shows the variety-dependent and seed-borne bacterial community assembly in roots of two choy sum varieties under CIP stress wherein the HAV prefers enriching Bacillaceae (*Bacillus* dominated), relative to the LAV that tends to enrich the Lachnospiraceae and Enterobacteriaceae (Fig. 2b, c). Bacterial assembly in plant rhizocompartments is determined by several factors, including the plant characteristics, nutrition supply, and exogenous stress [62, 63]. Accordingly, the stochastic process of seed-borne bacterial community assembly in the LAV root in the control treatment was likely owing to the abundant nutrient supply derived from the root exudates, e.g., LMWOAs [22]. Conversely, CIP served as the selective stress on the microbial assembly in the stress treatment, which significantly increased the deterministic process of the seed-borne bacteria community assembly ($p < 0.05$), leading to the decrease of PGP bacteria (Enterobacteriaceae and Bacillaceae) and the increase of pernicious bacteria (Lachnospiraceae) in the LAV root microbiota. In this case, the LAV suffered from more CIP stress damage and the resultant loss of biomass and root length (Fig. 1), leading to less root CIP capture and subsequent accumulation in shoot.

It is different from the LAV that the community assembly of the seed-borne bacteria in the HAV root microbiota always presented a deterministic process regardless of CIP stress (Fig. S6), which was owing to the specific enrichment of *Bacillus* in the HAV root microbiota, with the RA up to 60% (Figs. 2b, c). The HAV root tended to secret higher concentrations of LMWOAs in the root exudates relative to the LAV

root, especially the maleic acid with up to twofold difference between the two varieties under 0–1 mg/L of CIP exposure [22]. It is noteworthy that maleic acid (10.8–24.8 mg/L, i.e., 64 ~ 213 μM) constituted the predominant component in the root LMWOAs of both varieties, accounting for 48.4–73.5% of the total LMWOAs concentrations (mg/L) [22]. Tartaric acid (2.7–14.1 mg/L, i.e., 18.3–93.9 μM) and acetic acid (0.41–3.8 mg/L, i.e., 6.8–70 μM) accounted for 12.5–48.7% and 1.5–25% of the total LMWOAs concentrations (mg/L), respectively. These three LMWOAs could possess powerful chemoattraction for the *Bacillus* [64, 65].

A chemotaxis assay showed that maleic acid showed a strong chemoattraction on the strain BpB13, with ~ 4.0 times higher bacterium colony-forming units (CFU) in the treatment than in the control (Fig. S13). However, tartaric acid and acetic acid failed to perform the chemoattraction on the strain BpB13. Accordingly, the HAV root can specifically recruit *Bacillus* via producing high concentrations of maleic acid, thus showing the deterministic process of the seed-borne bacterial community assembly. Notably, although the MIC of CIP against the BpB13 was determined to be 0.25 mg/L, this strain exhibited enhanced RA in the presence of HAV under CIP stress at concentrations of 0.2 and even 1 mg/L (Figs. 6c and S11). This also suggested that HAV promoted the survival and CIP resistance of the strain (BpB13), potentially mediated by malic acid (acting as a chemoattractant) from HAV secretion. The activation of BpB13, in turn, facilitated biomass and resultant CIP accumulation of HAV. These findings highlight the critical roles of plant–microbe interactions in facilitating mutual adaptation to environmental stress.

Specifically, the enrichment of seed-borne *Bacillus* in the HAV root also promoted increase of the other PGP bacteria (e.g., Enterobacteriaceae), inducing greater cooperative interaction in the bacterial community network, relative to the LAV (Figs. 2 and 4). Such a bacterial community associated with *Bacillus* could mitigate the CIP toxicity to the HAV, and avoid loss of biomass, especially the root length (Figs. 4c, 5a, b), by improving antioxidase activities and decreasing MDA contents (Fig. 6a, b) in the HAV. Thus, a more robust root system of the HAV is conducive to root capture and subsequent accumulation of CIP (Figs. 4 and 5). In addition, the *Bacillus*-associated bacterial community can also promote the transpiration rate by improving expression of the aquaporin-associated genes and proteins in the plant [55–57], thus enhancing the uptake and translocation of CIP in the HAV via the transpiration stream. The inoculation experiments confirmed that the *Bacillus* strain BpB13 could significantly increase shoot accumulation and TF factors of CIP in the HAV (Fig. 5b). Therefore, the seed-borne bacterial community assembly plays an important role in regulating the CIP accumulation in various choy sum varieties.

Conclusion

This study highlights the pivotal role of seed-borne bacteria in shaping the root-associated microbial community and regulating the accumulation of organic pollutants in crops. It is demonstrated that HAV roots can produce high concentrations of maleic acid to specifically enrich *Bacillus* in the rhizosphere through both vertical and horizontal transmission pathways. The enriched *Bacillus* in plant roots secretes IAA and siderophores, which stimulate antioxidase activities in HAV, thereby enhancing its tolerance to CIP stress. Moreover, the enrichment of *Bacillus* promotes the development of a cooperative PGP bacterial community primarily through deterministic processes, which significantly improves biomass, particularly root length in HAV compared to LAV. This leads to increased root uptake and subsequent accumulation of CIP. The findings offer new insights into the mechanisms of organic pollutant accumulation in plants via plant-microbial interactions, addressing a substantial knowledge gap regarding the influence of seed-borne microbiomes. Additionally, the findings also elucidate the mechanism underlying the differential accumulation of CIP in edible parts among various plant varieties from the perspective of seed-borne bacteria.

Given the crucial role of plant-associated microbial community, especially seed-borne bacteria, in the accumulation of antibiotics in plants, future research should investigate the potential of microbial regulation as a means to mitigate agricultural pollution. Specifically, research could focus on screening and cultivating microbial strains that can effectively reduce antibiotic accumulation in plants or on modifying the composition of plant and soil microbial communities to minimize the accumulation of antibiotics and other organic pollutants in edible plant tissues. Additionally, it is worth noting that *Bacillus* has been used as a PGP bacterial supplement to improve crop yield. Considering its enhancing effect on CIP accumulation in the crop, further studies on the appropriate use of *Bacillus* are warranted to ensure the satisfactory harvest of agricultural products and low accumulation of pollutants in the edible parts of crops.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40168-025-02073-2.

Supplementary Material 1. Supplementary Information T1 Surface sterilization of choy sum seeds and roots. Supplementary Information T2 Sterility assessing of the axenic system. Supplementary Information T3 Gene sequencing and bioinformatics analysis. Supplementary Information T4 Extraction and analysis of CIP. Supplementary Information T5 Statistical analysis. Supplementary Information T6 Isolation and identification of root-associated bacteria. Supplementary Information T7 16S rRNA gene sequence of *Bacillus* strain BpB13. Supplementary Information T8 The representative *Bacillus* OTU sequences extracted from 16S rRNA gene amplicon sequencing data. Supplementary Information T9 Antimicrobial activity evaluation of CIP against BpB13. Supplementary Information T10 Determination of plant physiological characteristics. Supplementary Information T11 Identification of plant growth promoting properties. Supplementary Information T12 Chemotaxis assay. Supplementary tables. Table S1 Method of detection limits (MDLs) and the recoveries of CIP ($n=6$). Table S2 Summary of tests for differences in bacterial and fungal community composition with different CIP treatments between two varieties using PERMANOVA based on Bray–Curtis dissimilarity. Table S3 Correlations and topological properties of root-associated bacterial community co-occurrence networks of HAV and LAV under three CIP levels. Table S4 Sequence alignment results of the 16S rRNA gene between the representative *Bacillus* OTUs and the isolated *Bacillus* strain BpB13. Table S5 Comparison in plant growth promoting properties between the *Bacillus* bacterium isolated from HAV root in this study and the reported *Bacillus* bacteria. Table S6 Barcode sequences to split raw data of PRJNA1124639. Supplementary figures. Fig. S1 The assays of checking tissues surface sterilization. Fig. S2 Evaluation of the axenic system by plating the substrate solutions on TSA plates for 5 days of cultivation. Fig. S3 CIP concentration in nutrient solution grown HAV and LAV exposed to different levels of CIP (0.2 and 1 mg/L). Fig. S4 Relative abundances of dominant phyla of seed-borne bacteria (a) and fungi (b) communities in HAV and LAV exposed to different levels of CIP (0, 0.2 and 1 mg/L) grown in axenic system. Fig. S5 Nonmetric multidimensional scaling (NMDS) ordinations and PERMANOVA of seed-borne fungi communities of HAV and LAV exposed to different levels of CIP (0, 0.2 and 1 mg/L) grown in axenic system based on Bray–Curtis dissimilarity. Fig. S6 Assembly processes of seed-borne bacterial communities of HAV and LAV exposed to different levels of CIP (0, 0.2 and 1 mg/L) grown in axenic environment based on null model. Fig. S7 Co-occurrence network showing the correlations between CIP concentration and seed-borne bacterial communities of HAV (a) and LAV (b), respectively. Ratio of negative correlations between CIP concentration and major families of HAV and LAV in co-occurrence networks (c). Fig. S8 Correlations among the relative abundances of the differential core bacteria in HAV (a) and LAV (b). Fig. S9 Morphological observation of strain BpB13. Fig. S10 Phylogenetic tree derived from 16S rRNA gene sequences of strain BpB13 and related strains using maximum likelihood method. Fig. S11 Relative abundances of dominant OTU members of core *Bacillus* and dominant Bacillaceae under axenic environment with plant. Fig. S12 Antibiogram of the isolated *Bacillus* strain against CIP. Fig. S13 Chemoattraction of main LMWOA components (100 μM) in root exudates of the two choy sum varieties on the *Bacillus* strain BpB13. Fig. S14 Effects of IAA (100 μM) on CIP bioaccumulation in the two choy sum varieties.

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Authors' contributions
Yi-Ze Wang, Hai-Ming Zhao, and Xian-Pei Huang conceived and designed the experiments. Yi-Ze Wang, Yu Zhang, and Jin-Cheng Ye, carried out the experiments. Yi-Ze Wang, Nai-Xian Feng, Yan-Wen Li, and Bai-Lin Liu contributed to the data analysis and interpretation of the results. Yi-Ze Wang took the lead in writing the manuscript with the support from Lei Xiang, Ce-Hui Mo, Hai-Ming Zhao, Qing X. Li, and Quan-Ying Cai. All authors edited the manuscript and agree to the final submitted version.

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Data availability
Sequence data were deposited into NCBI Sequence Read Archive (SRA) under the accession number PRJNA1124639 and PRJNA1184200.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Guangdong Provincial Research Center for Environment Pollution Control and Remediation Materials, College of Life Science and Technology, Jinan University, Guangzhou 510632, China. [2]National-Regional Joint Engineering Research Center for Soil Pollution Control and Remediation in South China, Guangdong Key Laboratory of Integrated Agro-environmental Pollution Control and Management, Institute of Eco-environmental and Soil Sciences, Guangdong Academy of Sciences, Guangzhou 510650, China. [3]Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, HI 96822, USA.

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