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RESEARCH ARTICLE

Characterization of plant growth-promoting traits of *Enterobacter* sp. and its ability to promote cadmium/lead accumulation in *Centella asiatica* L.

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Abstract

In the present study, we characterized the plant growth-promoting traits of *Enterobacter* sp. FM-1 (FM-1) and investigated its ability to promote growth and increase IAA, P, and Fe concentrations as well as Cd and Pb accumulation in *Centella asiatica* L. (*C. asiatica* L.) in upstream area (UA) soil and downstream area (DA) soil that we collected from Siding mine. The results demonstrated that FM-1 secreted IAA, produced siderophores, and had P-solubilization ability even under Cd exposure. IAA secretion reached a maximum of 108.3 ± 1.3 mg L^{-1} under Cd exposure at 25 mg L^{-1}. Siderophore production reached a maximum of 0.94 ± 0.01 under Cd exposure at 50 mg L^{-1}. Pot experiments indicated that FM-1 successfully colonized the roots of *C. asiatica* L. In both soils, inoculation with FM-1 decreased the pH in rhizosphere soil and increased the bioavailability of both Cd and Pb. In addition, inoculation with FM-1 increased the IAA, P, and Fe concentrations and simultaneously promoted both Cd and Pb accumulation in *C. asiatica* L. The Cd and Pb concentrations in leaves increased 1.73- and 1.07-fold in the UA soil and 1.25- and 1.11-fold in the DA soil, respectively. Thus, the Cd-resistant strain FM-1 presented excellent PGP traits and could facilitate Cd and Pb phytoremediation by *C. asiatica* L.

Keywords *Enterobacter* sp. · Cadmium · Indole-3-acetic acid · Siderophores · Phosphate-solubilization ability

Introduction

With the development and utilization of mineral resources, soil heavy metal (HM) pollution has become a great concern worldwide. Among HMs, cadmium (Cd), a nonessential element for both plants and humans, is considered to be the metal with the most adverse effects on human health and is associated with the soil-to-food chain (Xu et al. 2020). In addition, Cd has become one of the most important inorganic pollutants in China, affecting at least 1.3 × 10^5 ha of cultivated land, and the soil in 25 regions belonging to 11 provinces is affected by Cd contamination (Rafiq et al. 2014; Ran et al. 2020). Guangxi Province, which is known as the "hometown of nonferrous metals," has more than 6800 mines of various types. Mineral development has caused serious Cd pollution around these mining areas. Therefore, it is urgent to explore and develop an effective remediation strategy for Cd contamination of soil. Traditional treatment methods available for Cd-contaminated soil generally use physical, chemical, and physical-chemical approaches; however, these technologies have limitations, including high energy consumption and costs, and may cause secondary pollution (Yao et al. 2012; Sarwar et al. 2017; Kim et al. 2019). Phytoremediation is eco-friendly, low-cost, easy to perform, and relatively safe to the environment and has been considered a green technology for the remediation of HM-contaminated environments (Paz-Ferreiro et al. 2014; Tiwari et al. 2016).

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Several Cd hyperaccumulators have been identified and used in Cd phytoremediation, including *Sedum alfredii* H. (Yang et al. 2004), *Solanum nigrum* L. (Wei et al. 2005), *Bidens pilosa* L. (Sun et al. 2009), *Viola baoshanensis* (Liu et al. 2004), etc. However, environmental stresses are present in most phytoremediation systems, and the success of these systems is restricted by the slow growth, selectivity for certain HMs, and low biomass production of plants as well as the long time frames for remediation (Chen et al. 2016a). Therefore, in recent years, plant-associated bacteria and their interaction with plant hyperaccumulators in Cd-contaminated soil have been given increasing attention (Glick 2010; Rajkumar et al. 2012; Kryuchkova et al. 2014; Ullah et al. 2015). Microbial-associated phytoremediation is a whole restoration system that can fully utilize the advantages of both plants and microorganisms. Plant growth-promoting bacteria (PGPB) include plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting endophytes (PGPE) (Wu et al. 2018). PGPB attach to and colonize the rhizosphere or other parts of plants to form a special microenvironment and can effectively increase plant biomass (Chen et al. 2017; Corretto et al. 2020). In the rhizosphere microenvironment, plant roots, rhizosphere microorganisms, and their metabolites play an important role in controlling the transformation of HMs, which makes phytoextraction of HMs more efficient and cost-effective (Glick 2010; Glick 2012; He et al. 2013; Syed-Ab-Rahman et al. 2018). PGPB have plant-promoting characteristics, including the production of the phytohormones indole-3-acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase to reduce the synthesis of ethylene and siderophores to help plants acquire sufficient amounts of iron (Fe); in addition, PGPR have nitrogen fixation abilities and phosphate (P)-solubilization abilities to fix nitrogen and solubilize inorganic P in soil (Glick 2014; Habibi et al. 2014; Parsons et al. 2015; Złoch et al. 2016; Raymond et al. 2020). PGPR can also produce extracellular polymeric substances (EPS) to prevent HM entry into bacterial cells to alleviate HM stress (Etesami 2018; Kumar et al. 2020). Previous studies indicated that various bacterial strains, including the following genera: *Pseudomonas*, *Bacillus*, *Rhodococcus*, *Rhizobium*, *Mycobacterium*, *Rahnella*, *Raoultella*, *Enterobacter*, *Micrococcus*, *Serratia*, *Acinetobacter*, etc., have plant growth-promoting (PGP) traits (Ambrosini et al. 2012; He et al. 2013; Habibi et al. 2014; Kryuchkova et al. 2014; Xu et al. 2019). He et al. (2013) indicated that *Rahnella* sp. JN6 produced IAA, siderophores, and ACC and had P-solubilization ability, which helped to promote growth and HM accumulation in *Brassica napus*. Chen et al. (2017) indicated that *Pseudomonas fluorescens* Sasm05 produced IAA to promote growth and Cd accumulation in *Sedum alfredii* H. However, there are various PGPBs, and their functions are different from those of different plants in enhancing plant resistance and HM accumulation. Therefore, the PGP traits of PGPB may have multiple consequences in plant-microbial interactions.

Strain FM-1, which was identified as *Enterobacter* sp. (GenBank accession number MF664375), was isolated in HM-contaminated soil in our previous study, which displayed a high tolerance of Cd, Mn, and Pb. FM-1 grown in Luria-Bertani (LB) culture medium without Cd had multiple PGP traits, producing IAA (72.85 ± 0.62 mg L^{-1}) and siderophores (0.21 ± 0.01) and showing P-solubilization ability (143.33 ± 2.13 mg L^{-1}) (Yu et al. 2018). Cd hyperaccumulator *Centella asiatica* L. (*C. asiatica* L.) was identified in our previous study, and a field survey indicated that *C. asiatica* L. can survive in soils with relatively high Cd contamination levels (Liu et al. 2016). We examined the potential utility of FM-1 for the phytoremediation of Cd-contaminated soil by two types of *C. asiatica* L. (Li et al. 2018b). However, the following problems were not solved in our previous studies: (a) with induction by Cd^{2+}, can FM-1 show PGP traits? (b) Does FM-1 colonize the rhizosphere of *C. asiatica* L.? (c) What are the change trends of the IAA, P, and Fe concentrations in plants? (We hypothesized that FM-1 promotes growth and Cd accumulation in *C. asiatica* L. by secreting IAA and siderophores and has P-solubilization ability.)

Hence, in the present study, the objectives were to (a) characterize the PGP traits of FM-1 under Cd^{2+} induction, (b) observe the colonization of FM-1 in the rhizosphere of *C. asiatica* L., and (c) investigate the effects of FM-1 inoculation on growth of *C. asiatica* L. and HMs accumulation (d) and on IAA, P, and Fe concentrations in *C. asiatica* L. Overall, our study will help to provide a theoretical basis for PGPB-assisted phytoremediation of HM-contaminated soil.

Materials and methods

Cd^{2+} tolerance evaluation

To evaluate the Cd^{2+} tolerance of this strain, FM-1 bacterial cells freshly grown for 12 h from LB culture medium, which was contained tryptone (1.0%), NaCl (0.5%), and yeast extract (0.5%) and were separated (8000×*g*, 10 min, at 4 °C) and used as inoculum at a volume of 2.0% (v/v) in a 250-mL Erlenmeyer flask containing 100 mL of LB culture medium and various concentrations (0 (control), 25, 50, 75, 100, 150, and 200 mg L^{-1}) of Cd^{2+} (prepared with $CdCl_2$). The cells were incubated at 30 °C in a shaking incubator (150 r·min^{-1}). Samples were collected after a given incubation time of 0, 6, 12, 24, 36, 48, 60, and 72 h, and cell growth was measured for their OD_{600} values.

The tolerance index (TI) was used to measure the tolerance of FM-1 to Cd^{2+} and was determined using formula (1) by comparing the OD_{600} value under metal treatment with that under the control (Tang et al. 2020).

$$\mathrm{TI}\ (\%) = \left(\mathrm{OD}_{600}\ \text{under}\ \mathrm{Cd}^{2+}\ \text{induction}/\mathrm{OD}_{600}\ \text{of control}\right) \times 100 \quad (1)$$

The cells of FM-1 cultivated on LB culture medium (the concentration of Cd^{2+} was 0 and 150 mg L^{-1}) for 24 h at 30 °C were harvested by centrifugation at 8000×*g* for 15 min at 4 °C, and cell pellets were prepared as described by Naik et al. (2012). Changes in the bacterial cell surface morphology were observed by scanning electron microscopy (SEM) (Quanta 200 FEG, Oxford Instruments, GB), and the qualitative elemental composition of the bacterial cell surface was analyzed by energy-dispersive spectroscopy (EDS) (D/Max 2500 DC, Rigaku D, Japan).

Characterization of PGP traits of FM-1

The PGP traits (IAA secretion, P solubilization, and siderophore production) of FM-1 were investigated at different Cd^{2+}- concentrations (0 (control), 25, 50, 75, 100, and 150 mg L^{-1}), reaction times (12, 24, 36, 48, 60, and 72 h), pH values (5.0, 6.0, 7.0. 8.0, and 9.0), and culture temperature (22, 26, 30, 34, and 38 °C). The IAA secretion of FM-1 was determined by the Salkowski reagent method described by Glickmann and Dessaux (1995). The P-solubilization ability of FM-1 was determined by growing FM-1 in modified Pikovskaya's medium described by Zaidi et al. (2006). Siderophore production of FM-1 was determined using the chrome azurol S agar (CAS) method described by Alexander and Zuberer (1991). The siderophore production ability was determined at 630 nm using formula (2).

$$\mathrm{SU} = [(A_r - A_S)/A_r] \quad (2)$$

where SU is the siderophore unit, A_r is the absorbance of the reference (CAS + noninoculated medium) at 630 nm, and A_S is the absorbance of the sample (CAS + cell-free sample supernatant) at 630 nm.

GFP-tagging and colonization of FM-1

FM-1 was transformed with the plasmid pET28a(+), which contained the gene for green fluorescent protein (GFP) under a constitutive promoter and the kanamycin resistance gene. Competent cells were prepared and precooled using 0.1 mol L^{-1} $CaCl_2$ and transformed by the heat shock method (Sharma et al. 2019). GFP-tagged FM-1 was observed using confocal laser scanning fluorescence microscopy (LSFM) (Olympus, BX63, Japan). GFP-tagged FM-1 cells were cultivated at 30 °C for 36 h in LB culture media consisting of 25 mg L^{-1} $CdCl_2$. FM-1 cells were collected and adjusted OD_{600} values to 0.25, 0.5, and 1.0. And the viable cells after being adjusted were 5.4×10^8, 1.4×10^9, and 3.4×10^9 CFU mL^{-1}, respectively, which were counted in LB agar.

Soil preparation and pot experiment setup

Soils were collected from two areas around the Siding Mine area, which were the upstream area (UA) and downstream area (DA). Their basic properties were provided in our previous research (Yu et al. 2020). Briefly, the properties of the UA soil were as follows: total Cd 17.93 mg kg^{-1}; bioavailable Cd 1.13 mg kg^{-1}; total Pb 879.16 mg kg^{-1}; bioavailable Pb 74.83 mg kg^{-1}; pH value 6.96; total nitrogen 1.73 mg g^{-1}; organic matter 17.28 mg g^{-1}; available P 6.72 mg kg^{-1}; nitrate nitrogen 1.43 mg kg^{-1}; and ammonia nitrogen 2.91 mg kg^{-1}. The properties of the DA soil were as follows: total Cd 166.56 mg kg^{-1}; bioavailable Cd 31.15 mg kg^{-1}; total Pb 5318.33 mg kg^{-1}; bioavailable Pb 311.45 mg kg^{-1}; pH value 6.56; total nitrogen 1.05 mg g^{-1}; organic matter 13.47 mg g^{-1}; available P 3.53 mg kg^{-1}; nitrate nitrogen 1.13 mg kg^{-1}; and ammonia nitrogen 0.89 mg kg^{-1}.

C. asiatica L. seedlings (3.50 ± 0.41 cm) were collected from the university campus which is located in Guilin, Guangxi Province, where the soil was not contaminated by Cd, Pb, or other HMs. Cd-Pb co-contaminated soil (2.5 kg) was equilibrated for 14 days. The seedlings of *C. asiatica* L. were transplanted and acclimatized for 14 days. A total of 30 mL of suspension bacteria (0 (control), 5.4×10^8, 1.4×10^9, and 3.4×10^9 CFU mL^{-1}) were applied to 2.5 kg soil (UA and DA soil, respectively), with inoculation concentration of 0 (control), 6.5×10^6, 1.7×10^7, and 4.1×10^7 CFU g $soil^{-1}$. The control group was applied with 30 mL LB medium. Plants were cultivated under natural light and watered daily to maintain a 70% moisture content. Meanwhile, nitrogen (50 mg kg^{-1}, ammonium sulfate), phosphorus (100 mg kg^{-1}, calcium phosphate), and potassium (40 mg kg^{-1}, potassium chloride) were applied in sufficient quantities for plant growth (Kamran et al. 2019).

Sample collection and analysis

Plants were harvested and collected after 45 days (from May 1, 2019 to June 15, 2019) and divided into two portions. One potion was dried and used to determine the metal concentration including Cd, Pb, and Fe, as mentioned by Yu et al. (2020). A standard sample (GSBZ 51001–94 ESP-1) was used to evaluate the accuracy of the procedure. The other fresh potion was used to determine the IAA and P concentrations. To assay the IAA concentration, fresh tissue samples were frozen in liquid nitrogen and ground into powder, and 10-mg samples were dissolved in 100 μL of 10 mmol L^{-1} phosphate-buffered saline (PBS, pH = 7.5) which contained 0.1% (w/v) gelatine and 0.1% (v/v) Tween 20, and then

centrifuged at 5000×*g* for 5 min; then, the supernatants were quantified using enzyme-linked immunosorbent assays (ELISAs, Mlbio, Shanghai, China) (Wang et al. 2020). The molybdenum blue colorimetric method was used to assay the P concentration according to (Lindner 1944). Briefly, 2.0 g fresh plant tissue samples were ground with a mortar and pestle and homogenized with 50 mL deionized water and quartz sand. The homogenate was placed in a 25-mL volumetric flask and centrifuged at 3000×*g* for 15 min. The supernatant was collected and measured at 660 nm.

Roots were collected and rinsed, after being cut with a scalpel; the root samples were placed on a glass slide; an appropriate amount of sterile water was dripped onto the samples; they were flattened slightly, and a cover glass was added to make a press (Lin et al. 2012). Root colonization was observed using a confocal laser scanning fluorescence microscope (LSFM) (Olympus, BX63, Japan).

Statistical analysis

Phytoextraction factor (PEC) and bioaccumulation factor (BAF) were calculated as the ratio between the Cd or Pb concentrations in the plant to the total or bioavailable Cd or Pb concentrations in soils, respectively. The translocation factor (TF) is the ratio between the Cd or Pb concentrations in stems and leaves to those in the roots. Data were tested at a significance level of $P < 0.05$ based on Duncan's multiple range test using one-way analysis of variance (ANOVA). Data are expressed as the means ± standard deviations (SDs).

Results

Evaluation of the Cd^{2+} tolerance of FM-1

FM-1 was able to grow in LB culture medium containing a relatively high concentration of Cd^{2+} (0-250 mg L^{-1}). There was a threshold Cd^{2+} concentration of 50 mg L^{-1}, below which the growth of FM-1 was similar to that of the control group with the addition of Cd^{2+}. However, when the concentration of Cd^{2+} was set above 150 mg L^{-1}, significant growth inhibition was observed. As shown in Figure 1A, the OD_{600} value of the culture broth containing Cd^{2+} (250 mg L^{-1}) was obviously lower than that of the control group. The TI values of FM-1 at different Cd^{2+} concentration levels are presented in Figure 1B. TI values showed a significant reduction at high Cd^{2+} concentrations, especially when Cd^{2+} concentrations > 50 mg L^{-1}. Specifically, when Cd^{2+} concentrations were 150 mg L^{-1}, the TI value still reached 65.5%, which indicated that FM-1 had a high Cd tolerance. However, when Cd^{2+} concentrations reached 250 mg L^{-1}, the TI value decreased to 43.1% after 48 h of cultivation.

SEM observation showed that in the culture medium without Cd^{2+}, the length of bacteria cells was between 0.890 and 1.26 μm, while the width of the cells was between 0.339 and 0.394 μm (Figure 1C). The original cells were smooth, stubby rods, and of uniform size. However, after Cd^{2+} (150 mg L^{-1}) adsorption, the length of the cells was between 0.881 and 1.14 μm, while the width of the cells was between 0.320 and 0.364 μm (Figure 1D). Most of the cells exhibited deformations. Additionally, FM-1 was subjected to Cd^{2+} stress, and the cells were surrounded by white flocculated sediments, which could be attributed to the EPS secreted by the bacterial cells forming complexes with Cd^{2+}. Further tests by EDS analysis (Figure 1C and D) provided information on the elements on the bacterial cell surface. As presented in Figure 1D, it was clear that there was a peak of Cd^{2+} between 2.75 and 3.95 keV. Moreover, the detected content and atomic ratio were significantly increased to 38.01% and 8.62% (Figure 1C), which indicated successful Cd^{2+} adsorption by FM-1 and confirmed that EPS can be secreted by FM-1 cells in the presence of Cd^{2+}.

Characterization of PGP traits of FM-1 under different culture conditions

The IAA and siderophore production and P-solubilization ability of FM-1 grown in culture media containing various concentrations of Cd^{2+} under different cultivation times, temperatures, and pH values are presented in Figure 2. When the Cd^{2+} concentration ranged from 0 to 25 mg L^{-1}, the presence of Cd^{2+} increased the secretion of IAA. The IAA concentration reached a maximum of 108.3 ± 1.3 mg L^{-1} when the Cd^{2+} concentration, cultivation time, pH, and temperature were 25 mg L^{-1}, 48 h, 7.0 and 30 °C, respectively (Figure 2A–C). However, when the Cd^{2+} concentration was higher than 75 mg L^{-1}, the IAA concentration decreased with increasing Cd^{2+} exposure (Figure 2A). For P-solubilization ability, under the same cultivation conditions, the P-solubilization ability of FM-1 decreased with increasing Cd^{2+} exposure, especially when the cultivation time was below 36 h (Figure 2D). Siderophore production was stimulated with Cd^{2+} exposure when the cultivation pH and temperature were 7.0 and 30 °C, respectively, irrespective of the cultivation time (Figure 2G). With increasing Cd^{2+} concentrations, siderophore production presented a trend of first increasing and then decreasing and reached a maximum of 0.94 ± 0.01 when the Cd^{2+} concentration, cultivation time, pH, and temperature were 50 mg L^{-1}, 48 h, 7.0, and 30 °C, respectively (Figure 2G–I). A neutral cultivation environment (pH = 7.0) was conducive to the P-solubilization ability and IAA and siderophore production of FM-1, and higher or lower pH values inhibited the secretion of PGP substances

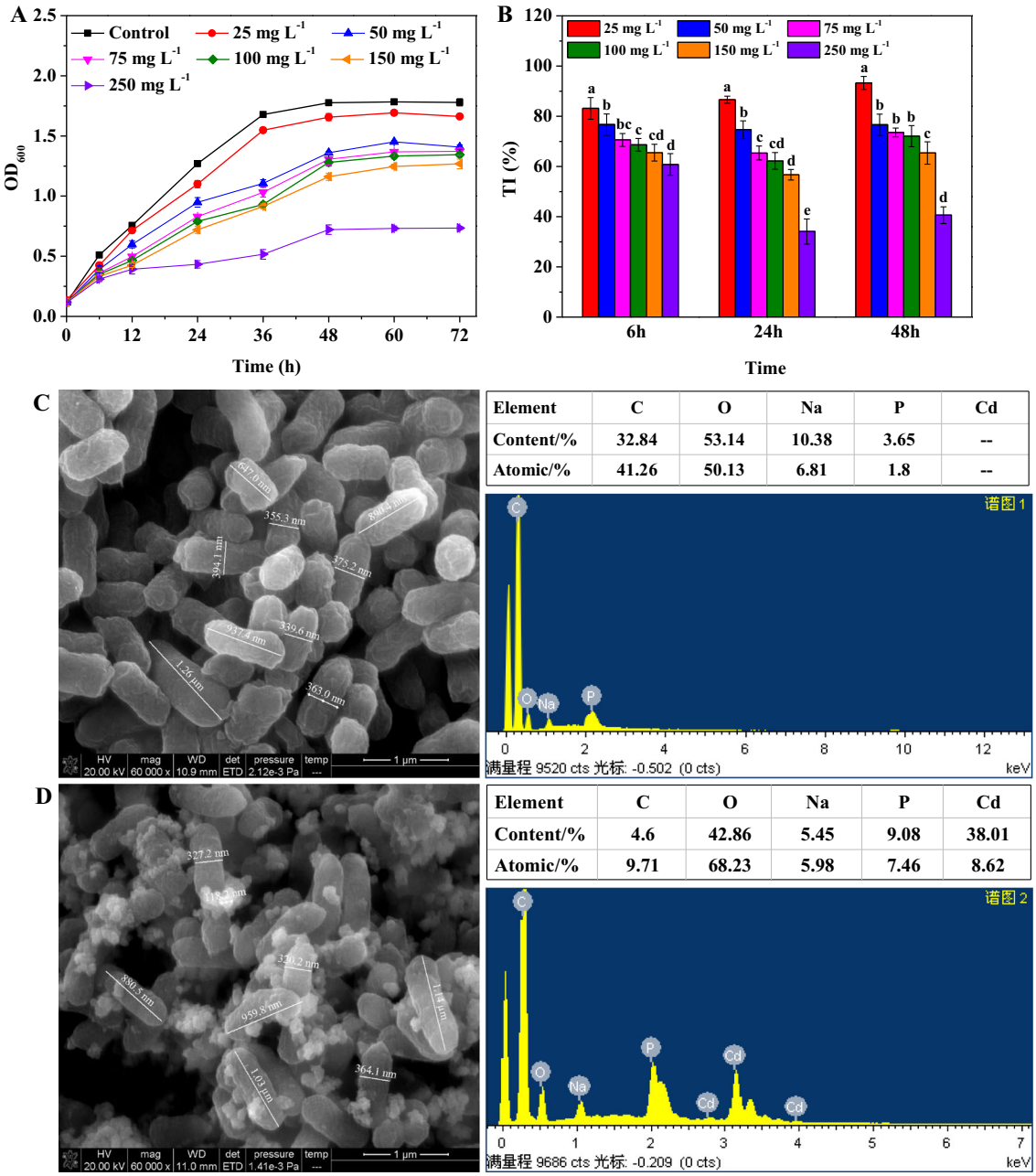


Fig. 1 *Enterobacter* sp. FM-1 growth during 72 h of incubation (**A**) in different concentrations of Cd2+, TI values (**B**), SEM, and EDX analyses of *Enterobacter* sp. FM-1 cultivated with (**C**) and without (**D**) Cd2+. The error bars represent the SD ($n = 3$)

(Figure 2B, E, and H), which indicated that the pH of the culture medium and the PGP traits of FM-1 were closely related. Specifically, when the cultivation pH was 9.0, the production of siderophores was significantly suppressed (Figure 2H). The cultivation temperature of 30 °C was the most suitable temperature for the production of IAA and siderophores by FM-1 (Figure 2C and I). However, a higher cultivation temperature of 34 °C was more suitable for promoting the P-solubilization ability of FM-1 (Figure 2F).

Promotion on the growth and HM accumulation of *C. asiatica* L. in inoculated FM-1

Colonization of FM-1 in the roots of *C. asiatica* L. and impact on plant growth

FM-1 was successfully tagged by GFP as shown in Figure 3B. In both soils, LSCM showed that under different inoculation levels, GFP-tagged FM-1 successfully colonized the roots of *C. asiatica* L. (Figure 3C and D). In both soils, FM-1

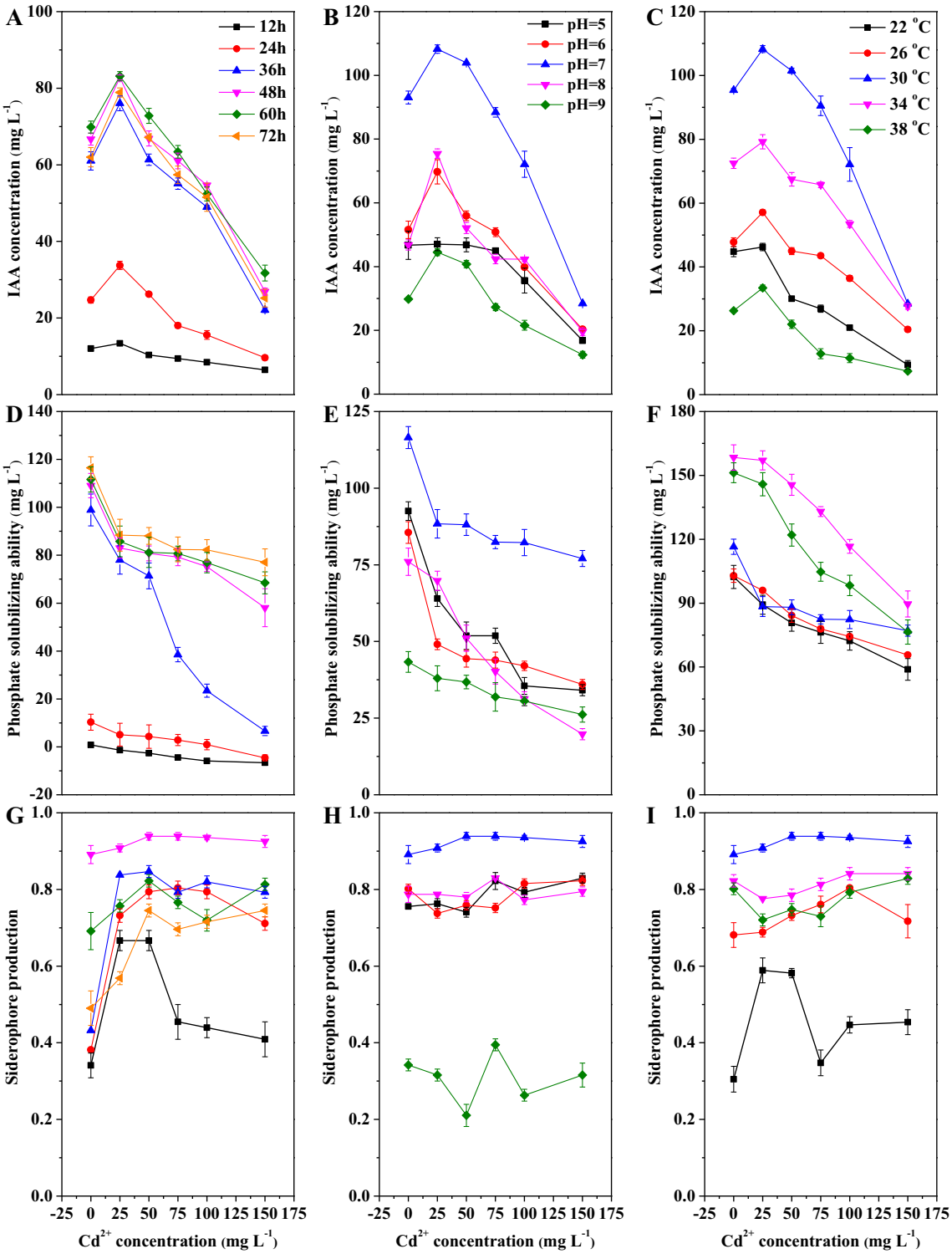


Fig. 2 IAA secretion, phosphate-solubilization ability, and siderophore production of *Enterobacter* sp. FM-1 under different initial Cd2+ concentrations at different reaction times (**A**, **D**, and **G**), pH levels (**B**, **E**, and **H**), and temperatures (**C**, **F**, and)). The error bars represent the SD (n = 3)

inoculation increased the biomass of *C. asiatica* L. (Figure 3E and F). Both height and weight of *C. asiatica* L. were significantly increased when inoculated with FM-1 (≥6.5 × 10^6 CFU g $soil^{-1}$) compared to noninoculated control. Additionally, both the height and weight increased with increasing FM-1 levels and reached the highest with 4.1 × 10^7 CFU g $soil^{-1}$ FM-1, which increased by 2.35- and 4.27-fold in UA soil and 2.53- and 4.55-fold in DA soil, respectively.

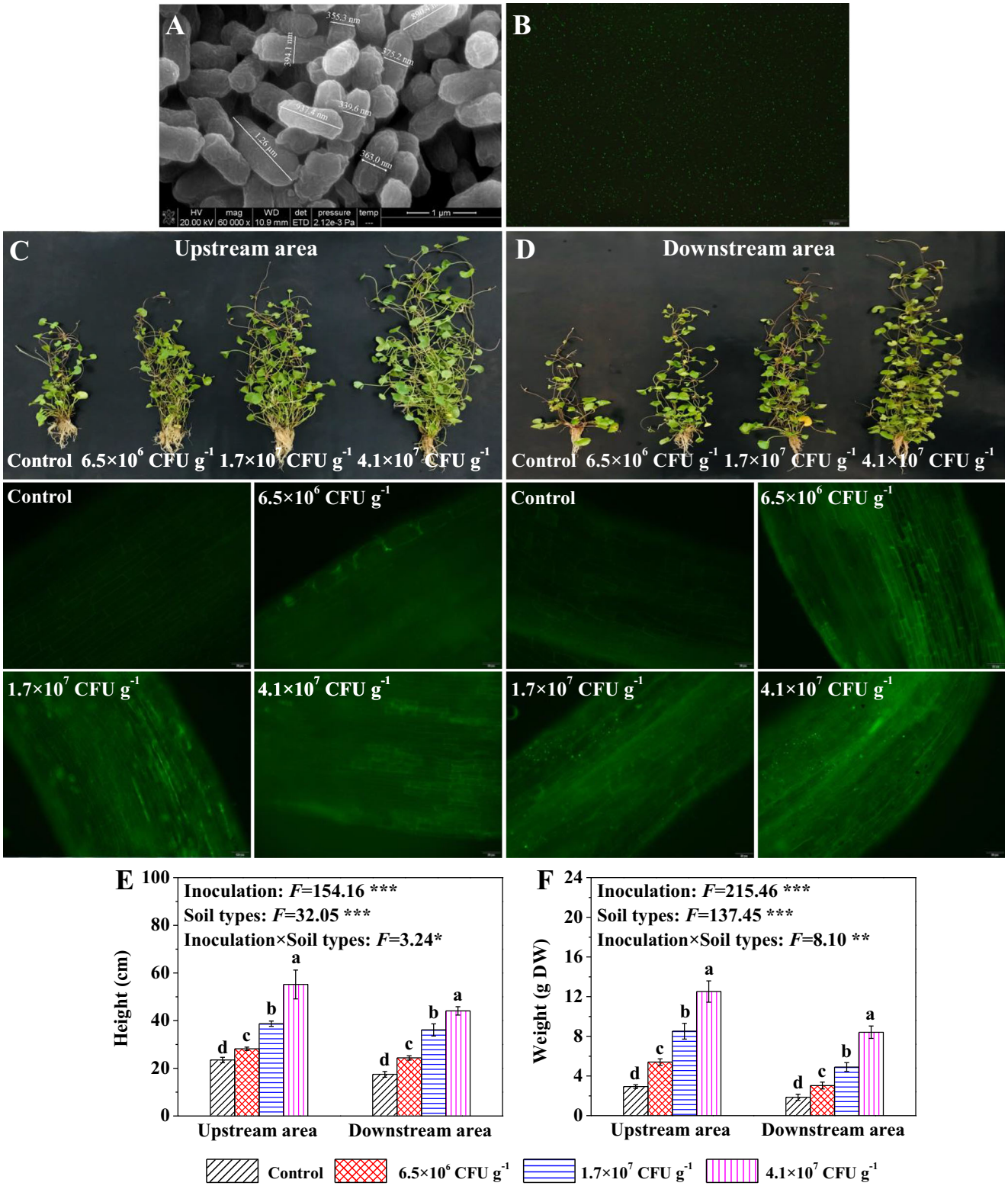


Fig. 3 SEM of *Enterobacter* sp. FM-1 (**A**) and LSFM (**B**) of GFP-*Enterobacter* sp. FM-1. GFP-FM-1 colonized the roots of *C. asiatica* L. under different bacterial inoculation concentrations in upstream area soil (**C**) and downstream area soil (**D**). Height (**E**) and weight (**F**) of *C. asiatica* L. under different bacterial inoculation concentrations. The weight is expressed on the basis of tissue dry weight (DW). The error bars represent the SD ($n = 6$). The different lowercase letters above the bars in the graph denote significant differences at $P < 0.05$ between the different bacterial inoculation concentrations in the same plant or tissues based on Duncan's multiple range test. The results of two-way ANOVAs are shown in the text; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$

Soil pH and bioavailability of Cd and Pb

Compared to the noninoculated control, in both soils, soil pH value significantly decreased by 2.7% in UA soil and 4.1% in DA soil, respectively, with the inoculation of FM-1 (≥6.5 × 10^6 CFU g $soil^{-1}$). Soil bioavailable Cd reached the highest with 4.1 × 10^7 CFU g $soil^{-1}$ FM-1, which increased by 1.32-fold and 1.21-fold in UA and DA soil, respectively. Soil bioavailable Pb reached the highest with 4.1 × 10^7 CFU g $soil^{-1}$ FM-1, which increased by 1.17-fold and 1.18-fold in UA and DA soil, respectively (Table 1).

IAA, P, and Fe concentrations in *C. asiatica* L.

Compared to the noninoculated control, IAA concentrations in the roots and stems significantly increased with the inoculation of FM-1 and reach to the highest with 4.1 × 10^7 CFU g $soil^{-1}$ FM-1, which increased by 1.42- and 1.14-fold in UA soil, and by 1.31- and 1.12-fold in DA soil, respectively. In addition, IAA concentrations in leaves were significantly increased by 1.16-fold and 1.23-fold in UA and DA soil, respectively. Two-way ANOVA results indicated that IAA concentrations in the tissues of *C. asiatica* L. were significantly influenced by FM-1 inoculation ($P < 0.001$) and soil type ($P < 0.001$). However, the interaction between them did not have a significant impact on the IAA concentrations in the plant tissues ($P > 0.05$) (Figure 4A).

Compared to noninoculated control, P concentrations in the roots, stems, and leaves of *C. asiatica* L. increased by 1.51-, 1.89-, and 1.41-fold in UA soil, and by 1.63-, 1.56-, and 1.79-fold in DA soil, respectively, with 4.1 × 10^7 CFU g $soil^{-1}$ of FM-1 (Table 2). Two-way ANOVA results indicated that P concentrations in *C. asiatica* L. were significantly influenced by FM-1 inoculation ($P < 0.001$) and soil type ($P < 0.001$). Moreover, the interaction between them significantly influenced the P concentrations in roots and stems ($P < 0.01$ or $P < 0.001$), but there was no significant impact on the P concentration in the leaves ($P > 0.05$) (Figure 4B).

Fig. 4 IAA (**A**), P (**B**), Fe (**C**), Cd (**D**), and Pb (**E**) concentrations in the roots, stems, and leaves of *C. asiatica* L. under different bacterial inoculation concentrations. The IAA and P concentrations are expressed on the basis of tissue fresh weight (FW), and the Fe, Cd, and Pb concentrations are expressed on the basis of tissue dry weight (DW). The error bars represent the SD ($n = 6$). The different lowercase letters above the bars in the graph denote significant differences at $P < 0.05$ between the different bacterial inoculation concentrations in the same tissues based on Duncan's multiple range test. The results of two-way ANOVAs are shown in the text; *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$

Compared to noninoculated control, Fe concentrations reached the highest with 4.1 × 10^7 CFU g $soil^{-1}$ of FM-1, which increased by 2.96-, 2,84-, and 1.98-fold in UA soil, and by 1.38-, 2.45-, and 1.59-fold in DA soil, respectively, in the roots, stems, and leaves of *C. asiatica* L. Specifically, roots' Fe concentrations were much higher than those in stems and leaves in both UA and DA soils. Two-way ANOVA results indicated that Fe concentrations in the tissues of *C. asiatica* L. were significantly influenced by FM-1 inoculation ($P < 0.001$) and soil type ($P < 0.001$). Moreover, the interaction between them significantly impacted the Fe concentrations in the plant tissues ($P < 0.05$ or $P < 0.001$) (Figure 4C).

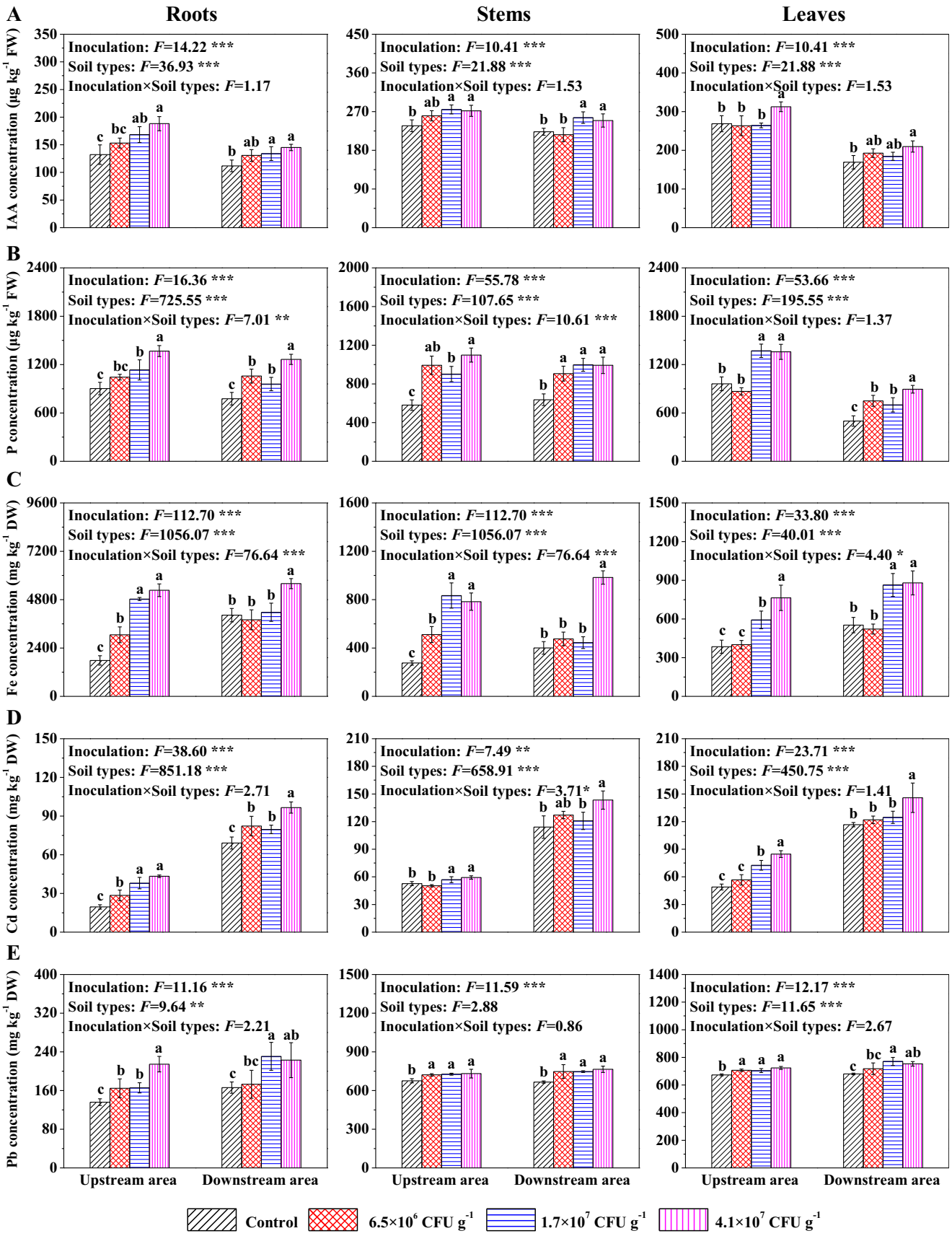
Cd and Pb accumulation in *C. asiatica* L.

For Cd accumulation in *C. asiatica* L., in UA soil, compared to the noninoculated control, Cd concentration significantly increased with the increase of FM-1 inoculation level and reach the highest with 4.1 × 10^7 CFU g $soil^{-1}$ of FM-1, which increased by 2.22-, 1.13-, and 1.73- fold, respectively, in the roots, stems, and leaves. In DA soil, compared to the noninoculated control, Cd concentrations reach the highest with 4.1 × 10^7 CFU g $soil^{-1}$ of FM-1, which increased by 1.39-, 1.25-, and 1.25-fold, respectively, in the roots, stems, and leaves. Two-way ANOVA results indicated that Cd concentrations in the tissues of *C. asiatica* L. were significantly influenced by FM-1 inoculation ($P < 0.01$ or $P < 0.001$) and soil type ($P < 0.001$). The interaction between them only

Table 1 Levels of bioavailable Cd and Pb and pH values in the presence of *C. asiatica* L. in Cd-Pb co-contaminated soil under different bacterial inoculation concentrations

Inoculated level	pH values		Bioavailable Cd concentration		Bioavailable Pb concentration	
	Upstream area	Downstream area	Upstream area	Downstream area	Upstream area	Downstream area
Uninoculated control	6.94 ± 0.03 a	6.59 ± 0.02 a	1.16 ± 0.03 c	29.49 ± 2.14 b	74.64 ± 1.73 b	315.00 ± 3.61 b
6.5 × 10^6 CFU g^{-1} (soil)	6.86 ± 0.02 b	6.39 ± 0.02 b	1.38 ± 0.04 b	30.19 ± 1.06 b	84.17 ± 3.03 a	349.7 ± 15.21 a
1.7 × 10^7 CFU g^{-1} (soil)	6.74 ± 0.03 c	6.37 ± 0.02 b	1.46 ± 0.04 a	33.02 ± 0.60 a	86.50 ± 5.32 a	354.67 ± 13.77 a
4.1 × 10^7 CFU g^{-1} (soil)	6.75 ± 0.03 c	6.32 ± 0.01 c	1.53 ± 0.05 a	35.79 ± 1.60 a	87.85 ± 2.83 a	369.20 ± 4.06 a

The means and the SD ($n = 6$) followed by a different lowercase letter within the same column denote a significant difference at $P < 0.05$ among different bacterial inoculation concentrations


A
Roots
Stems
Leaves
IAA concentration (μg kg-1 FW)
Inoculation: F=14.22 ***
Soil types: F=36.93 ***
Inoculation×Soil types: F=1.17
Inoculation: F=10.41 ***
Soil types: F=21.88 ***
Inoculation×Soil types: F=1.53
Inoculation: F=10.41 ***
Soil types: F=21.88 ***
Inoculation×Soil types: F=1.53
B
P concentration (μg kg-1 FW)
Inoculation: F=16.36 ***
Soil types: F=725.55 ***
Inoculation×Soil types: F=7.01 **
Inoculation: F=55.78 ***
Soil types: F=107.65 ***
Inoculation×Soil types: F=10.61 ***
Inoculation: F=53.66 ***
Soil types: F=195.55 ***
Inoculation×Soil types: F=1.37
C
Fe concentration (mg kg-1 DW)
Inoculation: F=112.70 ***
Soil types: F=1056.07 ***
Inoculation×Soil types: F=76.64 ***
Inoculation: F=112.70 ***
Soil types: F=1056.07 ***
Inoculation×Soil types: F=76.64 ***
Inoculation: F=33.80 ***
Soil types: F=40.01 ***
Inoculation×Soil types: F=4.40 *
D
Cd concentration (mg kg-1 DW)
Inoculation: F=38.60 ***
Soil types: F=851.18 ***
Inoculation×Soil types: F=2.71
Inoculation: F=7.49 **
Soil types: F=658.91 ***
Inoculation×Soil types: F=3.71*
Inoculation: F=23.71 ***
Soil types: F=450.75 ***
Inoculation×Soil types: F=1.41
E
Pb concentration (mg kg-1 DW)
Inoculation: F=11.16 ***
Soil types: F=9.64 **
Inoculation×Soil types: F=2.21
Inoculation: F=11.59 ***
Soil types: F=2.88
Inoculation×Soil types: F=0.86
Inoculation: F=12.17 ***
Soil types: F=11.65 ***
Inoculation×Soil types: F=2.67
Upstream area
Downstream area
Control
6.5×10^6 CFU g^-1
1.7×10^7 CFU g^-1
4.1×10^7 CFU g^-1

significantly impacted the Cd concentration in the leaves of *C. asiatica* L. ($P < 0.05$) (Figure 4D).

For Pb accumulation in *C. asiatica* L., in UA soil, compared to the noninoculated control, Pb concentrations significantly increased with the inoculation of FM-1 (≥6.5×10^6 CFU g soil^{-1}), which increased by 1.58-, 1.08-, and 1.07-fold, respectively, in the roots, stems, and leaves, with 4.1×10^7 CFU g soil^{-1} of FM-1. In DA soil, Pb concentrations in *C. asiatica* L. significantly increased with the inoculation of FM-1, which increased by 1.32-, 1.15-, and 1.11-fold, respectively, in the roots, stems, and leaves, under inoculation with 4.1×10^7 CFU g soil^{-1} of FM-1. Two-way ANOVA results indicated that the Pb concentrations in plant tissues were significantly influenced by FM-1 inoculation ($P < 0.001$). However, soil type only significantly impacted the Pb concentrations in the roots and leaves ($P < 0.01$ or $P < 0.001$), and there was no significant impact on the Pb concentrations in the stems ($P > 0.05$). Moreover, the interaction between them did not have a significant impact on the Pb concentrations in the plant tissues ($P > 0.05$) (Figure 4E).

Phytoremediation performance of *C. asiatica* L.

Compared to the noninoculated control, PEC-Cd value significantly increased with 4.1×10^7 CFU g soil^{-1} of FM-1, which increased by 1.53-fold in UA soil and 1.29-fold in DA soil, respectively. Meanwhile, BAF-Cd value significantly increased with 4.1×10^7 CFU g soil^{-1} of FM-1, which increased by 1.17-fold in UA soil. However, FM-1 inoculation did not have a significant impact on the BAF-Cd value and TF-Cd value in DA soil ($P > 0.05$). In DA soil, compared to the noninoculated control, PEC-Pb value and BAF-Pb value significantly increased with 1.7×10^7 CFU g soil^{-1} FM-1, which increased by 1.18- and 1.05-fold, respectively. However, in UA soil, inoculation did not have a significant impact on the BAF-Pb values ($P > 0.05$). In addition, in DA soil, FM-1 inoculation did not have a significant impact on the TF-Pb values ($P > 0.05$). Two-way ANOVA results indicated that the PEC, BAF, and TF values of both Cd and Pb were significantly altered by soil type ($P < 0.05$ or $P < 0.001$). The interaction between them only had a significant impact on the TF value of Cd ($P < 0.01$) and the PEC value of Cd ($P < 0.001$) (Table S1).

Discussion

Many strains of *Enterobacter* sp., such as *Enterobacter aerogenes* MCC 3092 (Pramanik et al. 2018), *Enterobacter cloacae* K7 (Kryuchkova et al. 2014), *Enterobacter* sp. EG16 (Chen et al. 2016b), *Enterobacter* sp. CBSB1 (Qiu et al. 2014), *Enterobacter cloacae* Y16 (Xu et al. 2020), and *Enterobacter* sp. C1D (Sharma et al. 2019), have been reported to have PGP traits and the ability to accumulate Cd and other HMs. In the present study, FM-1 demonstrated a relatively high Cd tolerance at 150 mg L^{-1} and secreted EPSs under Cd stress. Similarly, in our recent study, FM-1 also demonstrated a relatively high Pb tolerance at 600 mg L^{-1} and secreted EPSs under Pb stress (Li et al. 2021). Etesami (2018) pointed out that EPS production was one of the mechanisms of HM tolerance in bacteria, preventing HM entry into bacterial cells by nonspecific binding of HMs and EPSs. Generally, bacteria produce EPSs during HM stress conditions to achieve EPS-mediated HM resistance (Sharma et al. 2019), which was also observed in our study (Figure 1D). However, the relationship and mechanism between HM

Table 2 Phytoextraction, bioaccumulation, and translocation factors of *C. asiatica* L. in Cd-Pb co-contaminated soil under different bacterial inoculation concentrations

Inoculated level	Phytoextraction factor (PEC)		Bioaccumulation factor (BAF)		Translocation factor (TF)	
	Cd	Pb	Cd	Pb	Cd	Pb
Upstream area soil						
Uninoculated control	10.70 ± 0.90 c	1.74 ± 0.04 b	104.60 ± 5.65 bc	10.20 ± 5.50 a	5.23 ± 0.59 a	9.94 ± 0.58 a
6.5×10^6 CFU g^{-1} (soil)	12.20 ± 1.20 c	1.87 ± 0.06 a	97.97 ± 6.24 c	11.02 ± 5.68 a	3.82 ± 0.58 b	8.75 ± 1.00 a
1.7×10^7 CFU g^{-1} (soil)	14.46 ± 1.26 b	1.87 ± 0.05 a	114.79 ± 8.86 ab	9.86 ± 4.08 a	3.43 ± 0.28 b	8.66 ± 0.50 a
4.1×10^7 CFU g^{-1} (soil)	16.39 ± 0.41 a	1.91 ± 0.06 a	122.51 ± 1.61 a	10.82 ± 6.46 a	3.33 ± 0.19 b	6.81 ± 0.60 b
Downstream area soil						
Uninoculated control	1.64 ± 0.01 b	0.28 ± 0.01 c	19.83 ± 0.87 a	4.69 ± 0.16 b	3.35 ± 0.30 a	8.13 ± 0.65 a
6.5×10^6 CFU g^{-1} (soil)	1.87 ± 0.16 ab	0.31 ± 0.02 b	18.92 ± 1.02 a	4.80 ± 0.05 ab	3.04 ± 0.21 a	8.63 ± 1.48 a
1.7×10^7 CFU g^{-1} (soil)	1.80 ± 0.06 b	0.33 ± 0.01 a	18.58 ± 0.37 a	4.93 ± 0.10 a	3.08 ± 0.04 a	6.64 ± 0.73 a
4.1×10^7 CFU g^{-1} (soil)	2.11 ± 0.23 a	0.33 ± 0.01 a	19.17 ± 1.08 a	4.72 ± 0.18 ab	3.00 ± 0.33 a	6.94 ± 1.19 a

The means and the SD ($n = 6$) followed by a different lowercase letter within the same column denote a significant difference at $P < 0.05$ among different bacterial inoculation concentrations

induction and the production of EPSs will be investigated in our future study.

IAA is the major member of the auxin family and is involved in plant root initiation, cell division, and cell enlargement (Teale et al. 2006; Shokri and Emtiazi 2010); thus, IAA-producing PGPB can help to effectively enhance plant biomass, induce rooting and germination, and promote root hair growth and cotyledon cell expansion (Strader et al. 2010; Rostami and Azhdarpoor 2019). In the present study, a low level of Cd exposure (<25 mg L^{-1}) helped to stimulate IAA secretion; however, IAA secretion declined significantly with increasing Cd^{2+} concentrations. Moreover, neutral cultivation conditions were better for IAA secretion of FM-1 (Figure 2A–C). Normally, IAA is biosynthesized by tryptophan, and chorismite acts as an upstream precursor and is also involved in the biosynthesis of IAA (Chen et al. 2016a). Low levels of Cd exposure and neutral cultivation conditions can induce the expression of tryptone synthesis-related genes, which will promote the biosynthesis of IAA, but high levels of Cd exposure and unsuitable cultivation conditions might reduce the activity of the IAA synthetase system and consume the synthetic raw materials of tryptophan, possibly blocking the biosynthesis of IAA. This finding was consistent with Shokri and Emtiazi (2010), who pointed out that *Rhizobium* strains and *Paenibacillus* nonsymbiotic bacteria yielded higher concentrations of IAA in neutral cultivation media. P is one of the most important nutrient sources for plants; however, the P accumulated in soil tends to be relatively unavailable for plants (Raymond et al. 2020). Hence, seeking microorganisms to utilize these reserves of soil P is of great interest. Bacteria that have P-solubilization abilities can solubilize the soil organic P that plants are unable to use, which is a great way to facilitate P uptake by plants (Richardson 2001; Rowe et al. 2016). In our study, the P-solubilization ability of FM-1 was inhibited by Cd exposure; however, at low levels of Cd exposure (<75 mg L^{-1}) with a longer cultivation time (>48 h), FM-1 still presented excellent P-solubilization ability, which ranged from 79.2 to 88.3 mg L^{-1} (Figure 2D). A previous study indicated that *Enterobacter cloacae* B1 presented P-solubilization ability over a wide range of cultivation medium pH values and temperatures, from 4.0 to 9.0 and 20 to 40 °C, respectively (Borham et al. 2017). Moreover, Sánchez-Cruz et al. (2020) indicated that the maximum P-solubilization ability of *Enterobacter hormaechei* C2 reached 351.42 mg L^{-1} when the pH was 4.0. The same results were found in our study: bacteria presented better P-solubilization ability when the cultivation media pH was 7.0 and the temperature was 34 °C (Figure 2E–F). Fe is one of the most abundant metals found in microorganisms, and siderophore production is the most common strategy for bacterial acquisition of Fe under Fe-limited conditions (Chen et al. 2016a). A previous study indicated that some metals can stimulate siderophore production and thus induce competition for siderophore binding with Fe, which might cause Fe deficiency and metal toxicity in microbes (Dimkpa et al. 2008; Wichard et al. 2008). In the present study, we found that siderophore production of FM-1 was stimulated under Cd exposure (Figure 2G). Additionally, Dimkpa et al. (2008) found that Al^{3+}, Cd^{2+}, Cu^{2+}, and Ni^{2+} stimulated the production of siderophores by *Streptomyces* spp., while siderophore production also promoted the biosynthesis of auxin by chelating these metals with siderophores because chelation decreased the concentrations of free toxic metals. Similarly, Sinha and Mukherjee (2008) indicated that high Cd exposure stimulated the production of siderophores by *Pseudomonas aeruginosa* KUCd1. However, a high level of HM exposure inhibits the growth of microbes, and this was also observed in the present study. This can be explained by the production of siderophores, which may be a metal defense mechanism of microbes. Thus, FM-1 presents PGP traits, including IAA secretion, siderophore production, and P-solubilization ability, even under Cd exposure.

Rajkumar et al. (2012) noted that PGP traits confer an advantage to bacteria for niche colonization in the rhizosphere of plants against other competitors. In our study, FM-1 successfully colonized the rhizosphere of *C. asiatica* L., and FM-1 inoculation significantly increased both the weight and height of *C. asiatica* L. in UA and DA soil (Figure 3E–D). Moreover, both the height and weight of *C. asiatica* L. in UA soil were higher than those in DA soil, which might have been caused by the high concentrations of HM in DA soil, thus inhibiting the biosynthesis of IAA by FM-1. This can be explained by the results presented in Table S2, which indicated that the IAA concentration in roots was negatively correlated with the Bio-Cd and Bio-Pb in the soil, and the height and weight of *C. asiatica* L. were positively correlated with the root IAA concentration. IAA production by bacteria can help to change the bioavailability of HMs in the rhizosphere microenvironment, promote HM uptake (Ran et al. 2020; Chen et al. 2021), and dramatically enhance root growth (Patten and Glick 2002). Wang et al. (2017) indicated that IAA application helped to increase H^+-ATPase activity in the root plasma membrane of *Medicago sativa* L., which promoted H^+ secretion from root tips and decreased the pH of the rhizosphere. In addition, lowering the pH, chelating cations, and competing with phosphate for adsorption sites in the soil are the major mechanisms involved in organic acid production by P-solubilizing bacteria to solubilize inorganic phosphates (Rathi and Gaur 2016). Moreover, Gupta and Kumar (2016) pointed out that the dissolution of HMs by P-solubilizing bacteria is mainly carried out through various direct or indirect metabolic activities, such as the secretion of organic acids. Organic acids, including ketogluconic acid, gluconic acid, citric acid, oxalic acid, tartaric acid, succinic acid, etc., dissociate into protons and low-molecular-weight organic acids, which might cause changes in soil pH and redox potential to promote the dissolution of HMs in the soil (Kumar 2016). In

the current study, soil pH was decreased and the bioavailability of HMs was increased under the inoculation with FM-1 to varying degrees (Table 1). Previous studies indicated that siderophore production by bacteria can alleviate Cd-induced stress through reduced oxidative stress and ethylene stress and provide mineral nutrients for plant growth (Pramanik et al. 2018; Xu et al. 2020). In addition, siderophore production can change the bioavailability of HMs such as Cd in the rhizosphere by releasing organic acids (Dimkpa et al. 2008). Moreover, this might aid the combination of siderophores with HM ions to form a metal-siderophore chelate, improving the activity of HMs in the plant rhizosphere and hence increasing the accumulation of HMs (Złoch et al. 2016). Therefore, FM-1 inoculation into UA and DA soils increased the biomass of *C. asiatica* L. and decreased the rhizosphere soil pH value.

Generally, the TF values are greater than 1.0 (Baker 1981), and Cd and Pb concentrations in aerial parts are greater than 100 mg kg^{-1} and 1000 mg kg^{-1} (Baker and Brooks 1989) are two standards to judge the phytoremediation ability. In our study, *C. asiatica* L. exhibited excellent Cd accumulation ability, especially in DA soil, where the bioavailable Cd concentration was higher than that in UA soil (Figure 4D). Although *C. asiatica* L. also presented great Pb accumulation ability, there was not much difference between the UA and DA soils, although the bioavailable Pb concentration in DA soil was far higher than that in UA soil (Figure 4E). As a Cd hyperaccumulator, we found that the Cd concentration in the aerial parts of *C. asiatica* L. ranged from 73.4 to 390 mg kg^{-1} (Liu et al. 2016). Inoculation with a high concentration of FM-1 was more conducive to increased Cd accumulation in *C. asiatica* L. (Figure 4D). These results might be due to the Cd tolerance and PGP traits of FM-1 even under Cd exposure. FM-1 inoculation increased the IAA, P, and Fe concentrations in *C. asiatica* L. (Figure 4A–C). Specifically, IAA and P concentrations in *C. asiatica* L. in UA soil were higher than those in DA soil at the same FM-1 inoculation level because a low level of Cd exposure was more appropriate for the biosynthesis of IAA and stimulated the P-solubilization ability of FM-1 (Figure 2A–F). Moreover, we proposed that a low level of HMs might stimulate the expression of IAA biosynthesis-related genes and P accumulation-related genes in *C. asiatica* L. Kolbert et al. (2012) indicated that a low concentration of Cu^{2+} stimulated the expression of IAA biosynthesis-related genes, such as amino synthetase genes, anthranilate synthase genes, and tryptophan synthesis genes, in *Arabidopsis thaliana* L. Additionally, a previous study indicated that P-solubilizing microbes provided more mineral elements for absorption by plants and increased plant survival in severe environments by improving the nutritional status of the plant and increasing the ratio of nutrient element contents to HM contents to enhance the HM tolerance and accumulation abilities of hyperaccumulators (Li et al. 2018a). Brito et al. (2014) indicated that colonization by arbuscular mycorrhizal fungi significantly increased the P concentration in wheat tissues, and plants rely on ATP to extract Mn or form low-solubility P-Mn compounds, thereby reducing the toxic effect of Mn on plant tissues and increasing Mn accumulation. Similarly, the results of our study indicated that the P concentration in the roots of *C. asiatica* L. was significantly correlated with the metal concentrations in the roots (Table S2). In the current study, the Fe concentrations in *C. asiatica* L. in DA soil were higher than those of plants in UA soil at the same FM-1 inoculation level, which might be due to the reason we mentioned above—that Cd exposure stimulated siderophore production by FM-1 (Figure 2G–I). The correlation analysis presented in Table S2 shows that the Fe concentrations in the roots of *C. asiatica* L. were positively correlated with the Cd concentration in the roots, and the Fe concentrations in the stems and leaves of the plant were positively correlated with the metal concentrations in the stems and leaves. These results agree with those of several previous studies, which indicated that the improvement in the Fe status of plants can help to alleviate HM stress (Cohen and Garvin, 1998; Biyani et al. 2019; Guha et al. 2020). Fe serves as an antioxidant cofactor, which can help to defend against oxidative stress induced by HMs; moreover, the formation of iron plaques can help to prevent the loss of chlorophyll (Jeong and Connolly 2009; Muneer and Qureshi 2013). Hence, FM-1 inoculation increased the IAA, P, and Fe concentrations in *C. asiatica* L. and promoted the accumulation of Cd and Pb in the plant.

Conclusion

PGP traits of FM-1 were characterized in the current study, and the FM-1 inoculation on the growth, IAA, P, and Fe concentrations, HM accumulation in *C. asiatica* L. cultivated in Cd-Pb co-contaminated soil was investigated. Our results demonstrated that FM-1 demonstrated a relatively high Cd tolerance and had multiple PGP traits, including IAA secretion, siderophore production, and P-solubilization ability, even under Cd exposure. A low level of Cd exposure was more appropriate for the biosynthesis of IAA than higher levels and stimulated the P-solubilization ability of FM-1, while a high level of Cd exposure stimulated the siderophore production of FM-1. Pot experiments indicated that FM-1 successfully colonized the rhizosphere, and plants exhibited excellent phytoextraction and bioaccumulation abilities. Meanwhile, FM-1 inoculation increased the height and weight of plants in both soils, decreased the rhizosphere soil pH, and increased the bioavailability of HMs. Moreover, IAA, P, and Fe concentrations in plants were increased under FM-1 inoculation, which simultaneously promoted Cd and Pb accumulation in the plants. Hence, inoculation with FM-1 facilitated HM phytoremediation by *C. asiatica* L. In the future, taking into consideration the large areas of Cd and Pb co-

contaminated soil in China, the following aspects are valuable for further study: (a) depict the relationship and mechanism between HM induction and the production of EPSs by FM-1 in aqueous and soil phase. (b) Investigate the interaction between FM-1 and other restoration species. (c) Application of FM-1 in long-term field-scale bioremediation to obtain more information in order to develop the phytoremediation technique.

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Declarations

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Consent to participate Not applicable.

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