Environmental Science and Pollution Research (2019) 26:23679–23688
https://doi.org/10.1007/s11356-019-05661-6

RESEARCH ARTICLE

Effects of biochar-immobilized bacteria on phytoremediation of cadmium-polluted soil

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Received: 16 January 2019 / Accepted: 3 June 2019 / Published online: 15 June 2019

Abstract

This work is the first report of the ability of biochar-immobilized cadmium-resistant bacteria (CRB) on promoting the efficiency of cadmium phytoextraction by *Chlorophytum laxum* R.Br. The survival of CRB immobilized on biochar in cadmium-contaminated soil at a concentration of 75.45 mg kg^{-1} was studied. The results found that both CRB, namely *Arthrobacter* sp. TM6 and *Micrococcus* sp. MU1, can survive and grow in cadmium-contaminated soil. To study phytoextraction in the pot experiments, 2-month-old *C. laxum* was individually planted in cadmium-contaminated soil and divided into four treatments, including (i) untreated control, (ii) biochar, (iii) biochar-immobilized (BC) *Arthrobacter* sp., and (iv) BC-*Micrococcus* sp. The results found that biochar-immobilized CRB did not cause any effect to the root lengths and shoot heights of plants compared to the untreated control. Interestingly, inoculation of biochar-immobilized CRB significantly increased cadmium accumulation in the shoots and roots compared to the untreated control. In addition, the highest cadmium content in a whole plant, best phytoextraction performance, and greatest bioaccumulation factor was found in plant inoculated with BC-*Micrococcus* sp., followed by BC-*Arthrobacter* sp. In conclusion, inoculation of biochar-immobilized CRB enhanced cadmium accumulation and translocation of cadmium from the roots to shoots, suggesting further applying biochar-immobilized CRB in cadmium-polluted soil for promoting cadmium phytoextraction efficiency of ornamental plants.

Keywords Immobilized bacteria · Biochar · Cadmium-contaminated soil · *Arthrobacter* sp. · *Micrococcus* sp. · *Chlorophytum laxum*

Introduction

Agricultural areas in several countries, e.g., China, Thailand, have been contaminated with cadmium (Simmons et al. 2003; Liu et al. 2016). The study of Simmons et al. (2003) reported that the agricultural soil surrounding the zinc mine in Tak province, northern Thailand, is polluted with cadmium level as high as 284 mg kg^{-1}. Edible crop plants which are grown in contaminated soil can accumulate cadmium, thus passing cadmium to consumers via the food chain (Shute and Macfie 2006; Rizwan et al. 2017). Owing to the problem of cadmium-contaminated soil, the remediation of heavy metals deserves attention, in particular phytoremediation. Phytoremediation has been widely studied both in pot and field trial experiments because it is a cost-effective and environmentally friendly technology for removal of diverse contaminants from the environment (Ali et al. 2013).

Phytoremediation of heavy metal–contaminated soils mainly encompasses two strategies known as phytostabilization and phytoextraction processes (Mahar et al. 2016). However, phytoextraction efficiency of heavy metals is limited by phytotoxicity of heavy metals and low heavy-metal bioavailability in soil (Ali et al. 2013). Improving cadmium phytoextraction efficiency, increasing cadmium bioavailability and cadmium accumulation in plants by inoculating with some specific bacteria have been widely studied (Khonsue et al. 2013; Liu et al. 2015; Li et al. 2018). However, bacterial cells which are directly used for soil inoculation might encounter the toxicity of heavy metals and they must compete with the indigenous soil microflora. Cell immobilization can improve microbial resistance to stressful environments, protect cells against the indigenous soil microflora,

Responsible editor: Elena Maestri

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and promote the efficiency of bioremediation (Jézéquel et al. 2005; Dzionek et al. 2016). Biochar is carbon-rich porous material which is derived from burning biomass under conditions of limited oxygen known as pyrolysis. The benefits of biochar on agricultural and environmental aspects have been reported (Yavari et al. 2015). Due to the porous structure and high internal surface area of biochar, it can retain water and nutrients in soil and its surfaces provide a highly suitable habitat for microbial colonization (Ab Aziz et al. 2015). Therefore, biochar is considered to be a good carrier material for cell immobilization.

However, the study of biochar for bacterial cell immobilization in heavy-metal phytoremediation is still limited. Most research has studied the use of biochar for cell mobilization of organic pollutants, e.g., polycyclic aromatic hydrocarbons (Chen et al. 2012) and pesticides (Liu et al. 2017). Biochar application to soil has been widely accepted as an option to enhance pollutant removal, soil carbon sequestration, and soil amelioration (Oliveira et al. 2017). However, the high amounts of biochar application which are ranged from 0.5 to 2.0% (*w*/*w*) reduced the bioavailability of pollutants to microorganisms (Liu et al. 2017). Therefore, this study used a low amount of biochar as a carrier material for cell immobilization in order to support bacterial growth and protect bacterial cells from toxic environments. The survival of biochar-immobilized cadmium-resistant bacteria (CRB) in cadmium-contaminated soil was monitored. In the pot experiments, *Chlorophytum laxum* R.Br. or Siam lily which is popularly grown for its ornamental beauty was used for cadmium phytoremediation because the low possibility of cadmium accumulated in the harvested plants will be transferred into food chains and it also can beautify polluted environments.

Materials and Methods

Strains of cadmium-resistant bacteria, plant seedlings, and cadmium-contaminated soil

Arthrobacter sp. TM6 and *Micrococcus* sp. MU1, cadmium-resistant bacteria (CRB), which were isolated from rhizosphere soil and plant root collected from a cadmium-polluted area, Tak province, northern Thailand, were used in this study (Prapagdee et al. 2012; Prapagdee et al. 2013). *Chlorophytum laxum* R.Br. plants were propagated from the hypogeal part of the mature plants and planted in uncontaminated soil for 2 months before transplanting to cadmium-contaminated soil. The fresh weight, shoot, and tubular root lengths of 2-month-old *C. laxum* were approximately 42.52 ± 2.84 g, 20.2 ± 1.8 cm, and 9.0 ± 1.6 cm, respectively.

Cadmium-contaminated soil was collected at a depth of 0–20 cm from a highly-cadmium-polluted agricultural area at the Mae Tao subcatchment, northern Thailand (N 16° 40.593, E 098° 37.630). The important physical and chemical properties of soil were sandy loam soil texture; pH (1:1 w/*v* H_2O) 7.5; electrical conductivity (1:5) of 0.20 ± 0.02 mS cm^{-1}; cation exchange capacity of 9.81 ± 0.32 cmol kg^{-1}; 2.02 ± 0.34% organic matter; and 0.10 ± 0.02% total nitrogen, as previously reported by Rojjanateeranaj et al. (2017). To determine the concentrations of total cadmium and extractable cadmium (represented as bioavailable cadmium), the soil sample was digested with a mixture of 1:3 *v*/*v* concentrated nitric acid and concentrated hydrochloric acid (McGrath and Cunliffe 1985) and a mixture of diethylenetriamine pentaacetic acid (DTPA), triethanolamine, and calcium chloride (Faust and Christians 2000), respectively. Cadmium concentrations were measured by flame atomic absorption spectrophotometry (FAAS) (Varian spectra model AA240FS, USA) with the detection limit of cadmium at 0.01 mg L^{-1}. All samples were analyzed in triplicates, and a certified soil ERM-CC141 (European Reference Material, Belgium) and a reagent blank (without soil) were carried out to ensure the accuracy and precision in the analysis.

Preparation and characterization of biochar-immobilized CRB

Biochar of cassava stem (*Manihot esculenta* L. Crantz) was prepared in a 200-L char stove under anoxic condition at a temperature of 300 °C for 120 min followed by cooling overnight in the stove (Prapagdee et al. 2014). After oven-drying of the biochar at 80 °C for 1 h, the surface area, pore volume, and average pore size of the biochar were determined using the Brunauer–Emmett–Teller (BET) method (nitrogen gas and autosorb-l-C-8, Quantachrome Instruments, USA). In addition, the element compositions of biochar were also detected using a scanning electron microscope equipped with energy dispersive x-ray spectroscopy (SEM-EDX) (JSM-6400, JEOL, Japan). Cell suspensions of CRB, *Arthrobacter* sp. and *Micrococcus* sp., were individually cultured in M9 minimal medium (Difco, USA) containing 2% (*w*/*v*) biochar (Liu et al. 2017) (at the average diameter of 2.56 ± 0.04 mm) to give OD_{600} of ~ 0.1 and incubated at 150 rpm, 28 °C in a rotary shaker for 3 days. The bacterial cultures were filtrated through Whatman filter paper no. 4 and washed twice with 50 mM phosphate buffer (pH 7.0) to remove unattached bacterial cells (Prapagdee and Wankumpha 2017). Biochar-immobilized CRB were air-dried for 4 h before further use. The surface appearance of biochar before and after immobilization was observed under a SEM.

Survival of biochar-immobilized CRB in cadmium-contaminated soil

The viable cell number of biochar-immobilized CRB was enumerated by suspending in 50 mM phosphate buffer (pH

7.0) and vigorously shaking to detach the cells from biochar for 15 min, followed by spreading on Luria-Bertani (LB) agar plate (Difco, USA). A 0.2% (*w*/*w*) of each biochar-immobilized CRB was individually inoculated in the sterile cadmium-contaminated soil in a sterile 50-mL polypropylene tube and incubated at room temperature with shaking in the dark condition. Cadmium-contaminated soil with biochar-immobilized CRB was collected at 3, 5, 10, 15, and 20 days after inoculation. Soil samples were suspended in 50 mM phosphate buffer (pH 7.0) and shaken vigorously for 15 min. The number of viable cells in the soil was enumerated using the standard plate count technique.

Greenhouse experiments of cadmium phytoremediation by *C. laxum*

To evaluate the efficiency of biochar-immobilized CRB on cadmium phytoextraction by *C. laxum*, 3 kg of cadmium-contaminated soil was placed in 25-cm-diamater plastic pots and an individual healthy *C. laxum* was planted in each pot as previously described by the study of Prapagdee and Wankumpha (2017). This experiment was designed using completely randomized design into four treatments with three replicates, including (i) 2-month-old *C. laxum* planted in cadmium-contaminated soil (untreated control); (ii) 2-month-old *C. laxum* planted in cadmium-contaminated soil with 0.2% (*w*/*w*) biochar added; (iii) 2-month-old *C. laxum* planted in cadmium-contaminated soil inoculated with 0.2% (*w*/*w*) biochar-immobilized *Arthrobacter* sp. (BC-*Arthrobacter* sp.); and (iv) 2-month-old *C. laxum* planted in cadmium-contaminated soil inoculated with 0.2% (*w*/*w*) biochar-immobilized *Micrococcus* sp. (BC-*Micrococcus* sp.).

Each strain of biochar-immobilized CRB was applied into the soils only treatment (iii) and (iv) at the areas of plant root zone with approximately 10-cm depth and manually mixed well before planting. To ensure the presence of bacteria in soil, each biochar-immobilized CRB was repeatedly inoculated into the soil at 3 and 6 weeks, respectively, after planting. Experimental pots were randomly placed in the greenhouse with natural light and watered daily with the same volume of deionized water in the morning for 9 weeks. The average temperature in the greenhouse was 31.2 ± 0.4 °C and the average relative humidity was 66.8 ± 3.9%. Chemical fertilizer (formula 13-13-13) was applied in each pot at 0.18 ± 0.01 g every month.

Plant samples were carefully removed from the pots at 3, 6, and 9 weeks after planting and rinsed with running tap water, followed by several rinses with distilled water, and then soaked in 0.01 M hydrochloric acid for 5 s, followed by a distilled water rinse (Zhang et al. 2014). The roots and shoots of the plants were separated and oven-dried at 70 °C for 48 h and kept in a desiccator before weighing the dry biomass of the samples. The dried roots and shoots were ground and acid-digested. Cadmium concentrations in acid-digested plant samples were analyzed by FAAS. Soil samples around the plant root zone in each pot were collected at 3, 6, and 9 weeks after planting. The concentrations of total and bioavailable cadmium in the soil were determined according to the methods of McGrath and Cunliffe (1985) and Faust and Christians (2000), respectively, using FAAS as previously described above.

Data calculation

The cadmium concentrations in the soil and plant samples were used to determine the cadmium phytoextraction efficiency as described in three values. Firstly, the phytoextraction coefficient (PEC) is calculated by dividing the cadmium concentration in the whole plant by the total cadmium concentration in the soil surrounding the plant roots (Kumar et al. 1995). Secondly, the bioaccumulation factor (BAF) is estimated by calculating the ratio of cadmium concentration in the whole plant to the bioavailable metal concentration in the soil surrounding the plant roots (Khaokaew and Landrot 2015). Thirdly, translocation factor (TF) is the ratio of cadmium concentration in aboveground plant tissues (shoots) to that in underground plant tissues (roots) (Mattina et al. 2003).

Statistical analysis

The data were expressed as means and standard errors from the triplicate values. Mean values of all treatments were compared by analysis of variance (ANOVA) at 95% confidence. Duncan's multiple range test was performed to determine the statistical significance at p value less than 0.05 using the SPSS 18.0 software.

Results and discussion

Characteristics of biochar before and after bacterial cell immobilization

The results from BET analysis of biochar found that the surface area, pore volume, and average pore size of the biochar were 2.184 $m^2\ g^{-1}$, 7.762 × 10^{-4} $cm^3\ g^{-1}$, and 9.03 nm, respectively. The pore size of biochar was smaller than that of chitosan flake (19.15 nm) and it is classified in meso-pore size (the range between 2 and 50 nm) (Prapagdee and Wankumpha 2017). The element compositions of biochar, including carbon, oxygen, magnesium, phosphorous, calcium, and potassium, were 70.64 ± 4.56, 28.12 ± 3.82, 0.31 ± 0.12, 0.35 ± 0.37, 0.01 ± 0.06, and 0.55 ± 0.31%, respectively. SEM micrographs illustrate the external morphology of biochar before (Fig. 1a) and after immobilization with *Arthrobacter* sp. (Fig. 1b) and *Micrococcus* sp. (Fig. 1c). The numbers of

viable cells of *Arthrobacter* sp. and *Micrococcus* sp. which were adhered on biochar surface were 8.2 and 8.6 $\log_{10}$ CFU g^{-1} of biochar, respectively. Bacterial cells can colonize on biochar surface, particularly on rough surfaces. We can assume that biochar can serve as a habitat of bacterial cells. Biochar typically has a high surface area featuring many functional groups, a high cation exchange capacity, and high stability which can be applied as a carrier for bacterial cell immobilization (Liu et al. 2017).

Survival ability of biochar-immobilized CRB in cadmium-contaminated soil

To further apply biochar-immobilized CRB for cadmium phytoremediation, the ability of these bacteria to survive in cadmium-contaminated soil was studied. The concentrations of total cadmium and bioavailable cadmium in contaminated soil were 75.45 and 21.75 mg kg^{-1}, respectively. Figure 2 presents the viability of bacteria in cadmium-contaminated soil after inoculation with biochar-immobilized CRB in soil for 20 days. The results found that the numbers of viable cells of *Arthrobacter* sp. and *Micrococcus* sp. increased from 6.12 to 8.38 and 6.64 to 7.50 $\log_{10}$ CFU g^{-1}, respectively. Biochar assisted bacterial cells in cadmium-contaminated soil to withstand stress from cadmium toxicity. The growth of BC-*Arthrobacter* sp. in contaminated soil was higher than that of BC-*Micrococcus* sp. because *Arthrobacter* sp. has higher tolerance to cadmium toxicity than *Micrococcus* sp. (Prapagdee et al. 2012; Prapagdee et al. 2013). It should be noted that bacterial cells immobilized in biochar can proliferate and survive in cadmium-contaminated soil. This may be explained by cell immobilization improving bacterial cells' tolerance to toxic or stressful environments (Jézéquel et al. 2005; Dzionek et al. 2016). Additional reasons are that biochar can adsorb and retain nutrients in the soil as a nutrient-rich material and provide benign soil moisture and temperature to support microbial survival (Jiang et al. 2017; Chen et al. 2018b).

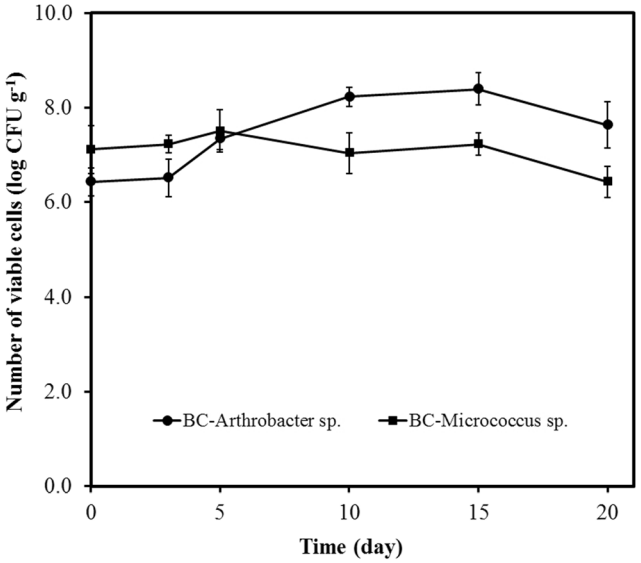

Fig. 2 Survival of biochar-immobilized CRB in cadmium-contaminated soil

Promoting of plant growth by biochar-immobilized CRB

The results from pot experiments in the greenhouse study found that *C. laxum* can grow in a highly-cadmium-contaminated soil (75.45 mg kg^{-1}). Figure 3 showed that the root dry weights of all treatments were higher than the shoot dry weights at all growth periods. The root dry weights of *C. laxum* inoculated with BC-*Micrococcus* sp. at 6 and 9 weeks were significantly higher than those of the untreated control by 1.2- and 1.1-fold, respectively. However, the weights of shoot dry biomass of *C. laxum* in all treatments were not significantly different ($p < 0.05$). In general, the typical morphology of the shoot of *C. laxum* has thin, slender, and elongated leaves; therefore, its size did not increase much within 9 weeks. Our previous study also reported that the root dry weight of *C. laxum* was increased in the presence of chitosan-immobilized *Micrococcus* sp. but not in the presence

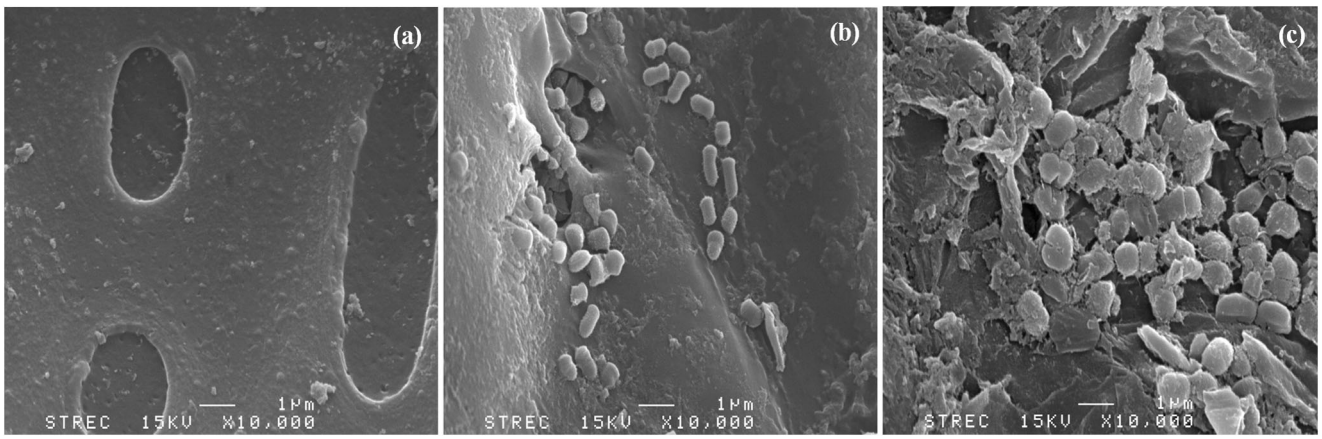

Fig. 1 Characteristics of **a** biochar, **b** BC-*Arthrobacter* sp., and **c** BC-*Micrococcus* sp. observed under SEM at × 10000 magnification

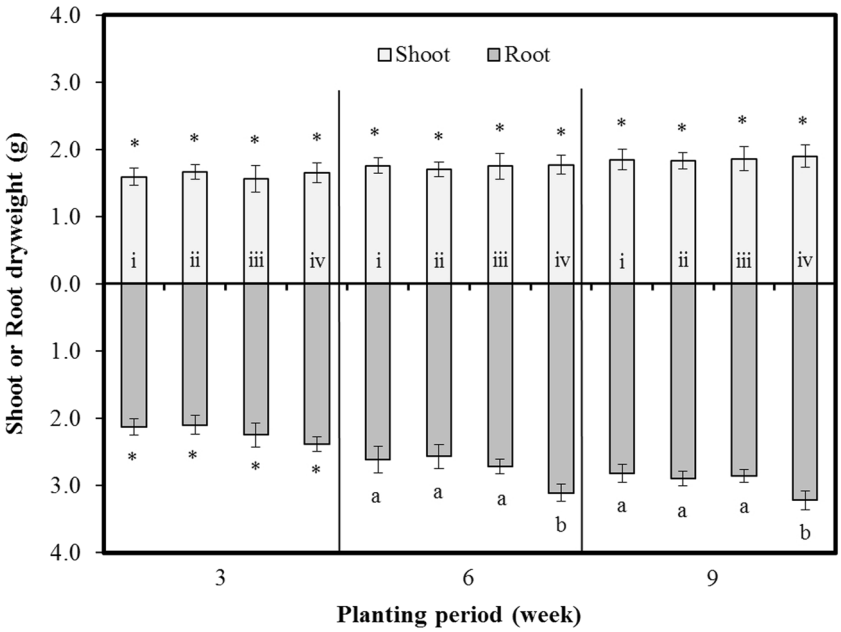

Fig. 3 Dry biomass weights of the roots and shoots of *C. laxum* after planting in cadmium-contaminated soil; (i) untreated control, (ii) biochar, (iii) BC-*Arthrobacter* sp., and (iv) BC-*Micrococcus* sp. The error bars are the SE ($n = 3$), and a different lowercase letter above a bar in the graph denotes a significant difference at $p < 0.05$ among different treatments at each planting period. An asterisk (*) above a bar in the bar graph denotes a non-significant difference at $p < 0.05$

of chitosan-immobilized *Arthrobacter* sp. (Prapagdee and Wankumpha 2017). Promotion of root growth of *C. laxum* by *Micrococcus* sp. involves a plant growth–promoting (PGP) trait of *Micrococcus* sp. in indole-3-acetic acid (IAA) production (Prapagdee et al. 2013). The plant growth–promoting activities of bacteria with PGP traits have been well documented in several publications. *Enterobacter aerogenes* and *Bacillus* sp. which have PGP traits enhance the growth of *Oryza sativa* L. and *Helianthus annuus* L. under cadmium toxic conditions (Pramanik et al. 2018; Siripan et al. 2018). In addition, our results found that biochar application and BC-*Arthrobacter* sp. inoculation did not promote the growth of *C. laxum* in cadmium-contaminated soil. Biochar can improve plant productivity by around 10% (*w*/*w*) (Liu et al. 2013), but it did not promote plant growth at low application rates.

Cadmium uptake and accumulation in C. laxum

After growing *C. laxum* in contaminated soil, cadmium accumulation in plant shoots and roots was determined and the results are presented in Fig. 4a and b, respectively. Cadmium contents in the shoots of *C. laxum* inoculated with either BC-*Arthrobacter* sp. or BC-*Micrococcus* sp. were higher than those of the untreated control at 6 and 9 weeks after planting (Fig. 4a). In particular, the sharp increase in cadmium accumulation in the shoots of *C. laxum* inoculated with either BC-*Arthrobacter* sp. or BC-*Micrococcus* sp. was found at 6 weeks after planting. Similar to the results of cadmium contents in the shoots, *C. laxum* inoculated with either BC-*Arthrobacter* sp. or BC-*Micrococcus* sp. had much more cadmium contents in the roots than that of untreated control at all planting periods (Fig. 4b). The highest cadmium contents in the roots were found at 9 weeks in *C. laxum* inoculated with either BC-*Arthrobacter* sp. or BC-*Micrococcus* sp. In comparison with the untreated control, cadmium accumulation in the roots of *C. laxum* inoculated with either BC-*Arthrobacter* sp. or BC-*Micrococcus* sp. at 9 weeks after planting increased by 1.8- and 1.8-fold, respectively. In addition, there was no significant difference ($p < 0.05$) of cadmium contents in the shoots and roots between the treatments with BC-*Arthrobacter* sp. and BC-*Micrococcus* sp. inoculation. It must be clearly stated that inoculation of both biochar-immobilized CRB significantly enhanced the cadmium accumulation in the shoots and roots of *C. laxum*. However, our previous study found that the performance of bacterial free cells on enhancing cadmium accumulation in *C. laxum* was a little bit better than that of immobilized cells, except at 9 weeks after planting, suggesting that immobilized cells need more time to adapt to proliferate compared to free cells because it is difficult to obtain nutrients to support their growth while immobilized on carrier material (Prapagdee and Wankumpha 2017). However, immobilization of cells protects microorganisms against an unfavorable environment and the indigenous soil microflora as well as easily to keep and apply in the fields.

Regarding the effect of biochar on cadmium uptake and accumulation, it was obviously seen that cadmium contents in the shoots and roots of untreated plant and *C. laxum* with biochar addition had no significant difference ($p < 0.05$), due to low amount of biochar application (0.2 wt%). Application of high amount biochar (1 to 15% *w*/*w*) in contaminated soil reduced cadmium toxicity to *Phaseolus vulgaris* L. growth (Hmid et al. 2015) and decreased cadmium uptake by *Sedum plumbizincicola* X.H. Guo et S.B. Zhou ex L.H. Wu (Lu et al. 2014). In addition, cadmium was retained in the

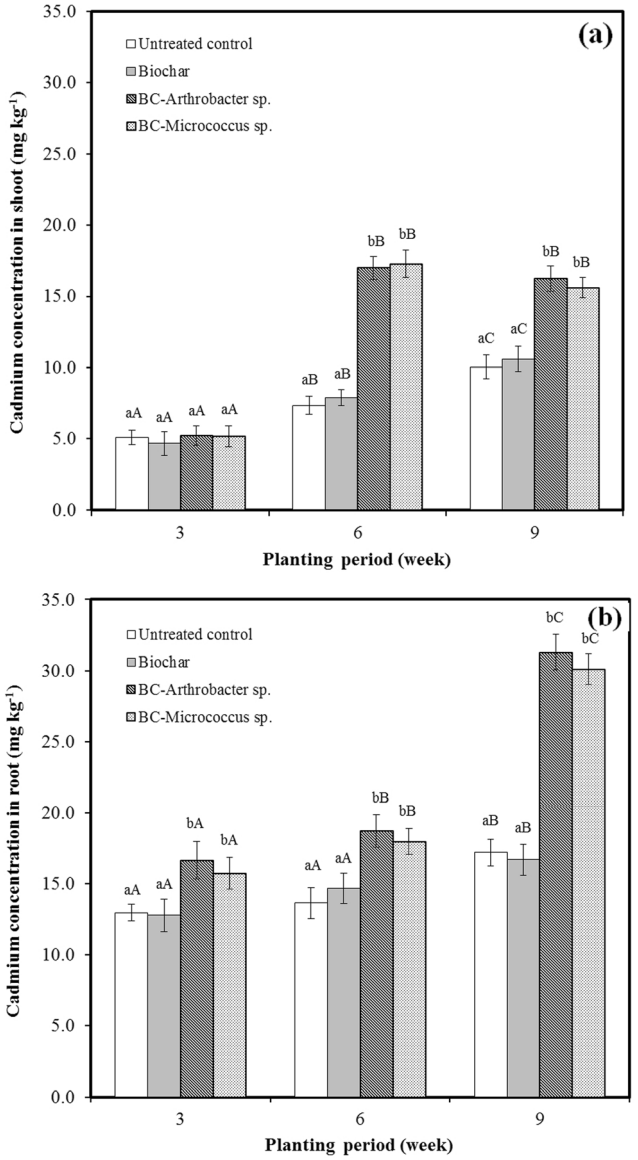

Fig. 4 Cadmium contents in **a** the root and **b** shoots of *C. laxum* planted in cadmium-contaminated soil. The error bars are the SE ($n = 3$), and a different lowercase letter above a bar in the graph indicates a significant difference at $p < 0.05$ among different treatments at each planting period. A different uppercase letter indicates a significant difference at $p < 0.05$ among different planting periods according to Duncan's multiple range test

roots more than in the shoots in all treatments and all growth periods. In *C. laxum* inoculated with BC-immobilized CRB, cadmium contents in the roots peaked at 9 weeks, while cadmium contents in the shoots peaked at 6 weeks after planting. These findings indicate that the translocation of cadmium from the roots to the shoots via xylem of *C. laxum* was limited when a certain toxic amount of cadmium was accumulated in the shoots. The explanation could be linked with cadmium precipitation and adsorption inside the plant root cells (Shute and Macfie 2006). However, inoculation of either BC-*Arthrobacter* sp. or BC-*Micrococcus* sp. increased the cadmium accumulation in the shoots by 2.3- and 2.4-fold, respectively, in comparison with the untreated plant at 6 weeks. Our findings suggest that a combined use of *C. laxum* and BC-immobilized CRB enhanced the efficiency of cadmium phytoextraction. This finding corresponds well to our previous studies (Prapagdee and Wankumpha 2017; Rojjanateeranaj et al. 2017). In addition, this observation was also corroborated by the work of Li et al. (2018) which claimed that *Enterobacter* sp. FM-1 increased cadmium uptake and accumulation in *Centella asiatica* L. leaves, stems, and roots compared to that in the uninoculated control plant.

Figure 5 depicts cadmium accumulation in a whole plant. Cadmium contents in whole plant increased with time in all treatments due to the progression of plant growth. Chen et al. (2018a) reported that the accumulation of heavy metals is positively related to plant biomass. Considering the effect of biochar-immobilized CRB inoculation, the highest cadmium content in a whole plant was found in plant inoculated with BC-*Micrococcus* sp., followed by plant inoculated with *Arthrobacter* sp. at all growth periods. In particular, at 9 weeks after planting, cadmium contents in a whole plant inoculated with BC-*Micrococcus* sp. and plants inoculated with *Arthrobacter* sp. were higher than that of a whole plant of untreated control by 1.9- and 1.8-fold, respectively. In addition, cadmium contents in a whole plant with biochar and in the untreated control were not significantly different ($p < 0.05$), indicating no effect of biochar addition on cadmium

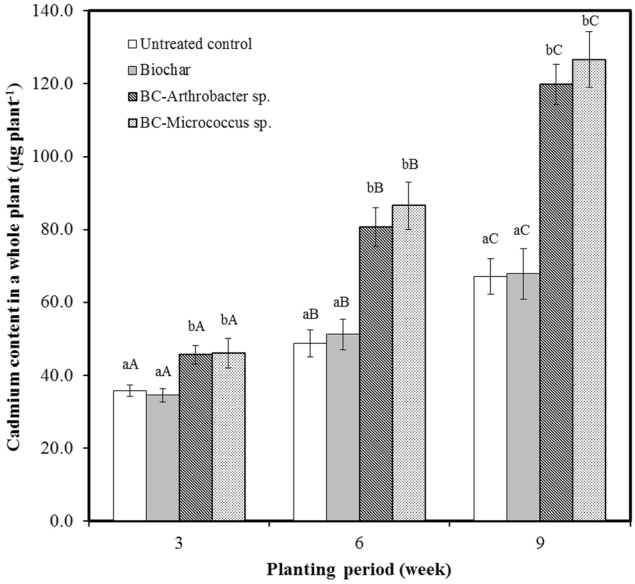

Fig. 5 Cadmium accumulation in a whole *C. laxum* planted in cadmium-contaminated soil. The error bars are the SE ($n = 3$), and a different lowercase letter above a bar in the graph indicates a significant difference at $p < 0.05$ among different treatments at each planting period. A different uppercase letter indicates a significant difference at $p < 0.05$ among different planting periods according to Duncan's multiple range test

uptake and accumulation in plant. Our findings suggest that a low amount of biochar application in cadmium-contaminated soil did not change cadmium bioavailability in soil and it can serve as a bacterial habitat for cell immobilization. Biochar application in the soil has positive effects on microbial groups and acts as a shelter for bacteria (Chen et al. 2012, 2018b; Yang et al. 2018); therefore, it is suitable to use as a natural carrier for bacterial immobilization.

We provide evidence that strongly suggests that inoculation of BC-*Arthrobacter* sp. or BC-*Micrococcus* sp. can speed up cadmium accumulation in the shoots and roots of *C. laxum*. This phenomenon could be clarified that *Arthrobacter* sp. and *Micrococcus* sp. can increase cadmium bioavailability in soil, resulting in increased cadmium uptake by plants. This explanation corresponds with that given by Rajkumar et al. (2010) who claimed that the inoculation of metal-mobilizing bacteria increases the process of phytoremediation. Several heavy metal–resistant bacteria, e.g., *Pseudomonas* sp., *Micrococcus* sp., *Klebsiella* sp., *Enterobacter* sp., and *Bacillus* sp., are also reported for their ability on increasing bioavailable heavy metals, promoting plant growth and enhancing heavy-metal phytoremediation in contaminated soil (He et al. 2009; Prapagdee et al. 2013; Li et al. 2018; Siripan et al. 2018). In contrast, some bacteria with PGP traits, e.g., *Serratia liquefaciens*, *Bacillus thuringiensis*, and *Enterobacter* sp., are able to increase plant growth and decrease metal uptake in plants by reducing metal bioavailability, depending on the bacterial species (Han et al. 2018; Mitra et al. 2018).

However, the bioavailable cadmium contents in soil in all treatments were not significant different ($p < 0.05$) (Table 1). It can be explained that plants can uptake bioavailable cadmium in soil, suggesting that more content of bioavailable cadmium had more plant uptake. The potential of these bacteria on increasing bioavailable cadmium in soil was corroborated by our previous work, reporting that the inoculation of *Arthrobacter* sp. and *Micrococcus* sp. free cells and chitosan-immobilized bacterial cells in cadmium-contaminated soil without plants increased the contents of bioavailable cadmium in soil compared to the uninoculated soil (Prapagdee and Wankumpha 2017). However, chitosan-immobilized cells were less able to promote cadmium bioavailability than free cells because free cells are in direct contact with cadmium on soil particles, while immobilized cells are adhered to the chitosan surface (Prapagdee and Wankumpha 2017). The possible mechanisms of *Arthrobacter* sp. and *Micrococcus* sp. on increasing cadmium bioavailable in soil and cadmium accumulation in plants involved the absorption of cadmium ions with the bacterial cell wall and exopolymers, resulting in increased cadmium bioavailability in soil (Prapagdee and Wankumpha 2017; Wiangkham and Prapagdee 2018).

Table 1 Bioavailable cadmium contents in contaminated soil after planting

Treatment	Bioavailable cadmium content (mg kg^{-1})*		
	3 weeks	6 weeks	9 weeks
Untreated control	13.44 ± 0.72	14.36 ± 0.60	14.45 ± 0.72
Biochar	13.75 ± 0.84	14.08 ± 1.16	14.15 ± 0.43
BC-*Arthrobacter* sp.	14.85 ± 0.74	15.78 ± 0.97	15.33 ± 0.54
BC-*Micrococcus* sp.	14.54 ± 0.53	15.55 ± 0.59	15.11 ± 1.04

An asterisk (*) indicates that there was no a significant difference at $p < 0.05$ among different treatments at each plantation period

Performance of cadmium phytoremediation

The three factors, i.e., phytoextraction coefficient (PEC), bioaccumulation factor (BAF), and translocation factor (TF), involved in the evaluation of cadmium phytoremediation performance of *C. laxum* are presented in Table 2. The results found that PEC values were quite low and slightly increased with time in all treatments. However, plant inoculated with biochar-immobilized CRB had higher PEC than that of the biochar treatment and the untreated control at all planting periods. A similar finding has been observed in our previous work (Prapagdee and Wankumpha 2017) which reported that the PEC of *C. laxum* inoculated with free cells and chitosan-immobilized cells of *Arthrobacter* sp. was 0.33. It should be realized that *C. laxum* had low PEC values. This seems to contradict with the study of Wang et al. (2012) who reported that *Chlorophytum comosum*, a spider plant, accumulates a large amount of cadmium in the shoot and root tissues and is claimed to be a cadmium-hyperaccumulating plant, indicating that different plant species have different cadmium phytoremediation efficiencies. Although *C. laxum* is not a hyperaccumulating plant, the inoculation of biochar-immobilized CRB significantly increased PEC.

In addition, the BAF values of *C. laxum* for all treatments ranged from 0.64 ± 0.05 to 1.72 ± 0.05. In comparison to the untreated control, the inoculation of either BC-*Arthrobacter* sp. or BC-*Micrococcus* sp. significantly increased BAF values, in particular at 9 weeks, with increases of 2.0- and 2.1-fold, respectively. The TF values of *C. laxum* added with biochar and the untreated control were low and slightly increased with time until 9 weeks. The TF values of plants inoculated with biochar-immobilized CRB peaked at 6 weeks and then decreased at 9 weeks after planting. It should be realized that at 9 weeks, *C. laxum* inoculated with BC-*Arthrobacter* sp. or BC-*Micrococcus* sp. had higher cadmium accumulation in the roots than that of plants at 6 weeks and cadmium contents in the shoot at 6 and 9 weeks were not too much different, resulting in decreasing TF values at 9 weeks. Cadmium is more retained in the root than in the shoot due to

Table 2 Phytoremediation performances of *C. laxum* with biochar-immobilized CRB inoculation and biochar treatment compared to the untreated control as expressed in terms of PEC, BAF, and TF after planting in the cadmium-contaminated soil

Treatment	PEC			BAF			TF		
	3 weeks	6 weeks	9 weeks	3 weeks	6 weeks	9 week	3 weeks	6 weeks	9 weeks
Untreated control	0.14 ± 0.02^{aA}	0.17 ± 0.02^{aA}	0.22 ± 0.03^{aB}	0.68 ± 0.09^{aA}	0.64 ± 0.05^{aA}	0.83 ± 0.06^{aB}	0.39 ± 0.03^{aA}	0.54 ± 0.09^{aB}	0.58 ± 0.07^{aB}
Biochar	0.14 ± 0.03^{aA}	0.16 ± 0.02^{aA}	0.21 ± 0.02^{aB}	0.69 ± 0.04^{aA}	0.77 ± 0.08^{aA}	0.98 ± 0.08^{aB}	0.37 ± 0.02^{aA}	0.54 ± 0.10^{aB}	0.63 ± 0.11^{aB}
BC-*Arthrobacter* sp.	0.18 ± 0.02^{aA}	0.25 ± 0.04^{bB}	0.38 ± 0.02^{bC}	0.77 ± 0.05^{bA}	1.24 ± 0.12^{bB}	1.67 ± 0.14^{bC}	0.31 ± 0.02^{aA}	0.91 ± 0.07^{bC}	0.52 ± 0.04^{aB}
BC-*Micrococcus* sp.	0.17 ± 0.03^{aA}	0.25 ± 0.02^{bB}	0.40 ± 0.04^{bC}	0.76 ± 0.07^{bA}	1.21 ± 0.08^{bB}	1.72 ± 0.05^{bC}	0.33 ± 0.01^{aA}	0.96 ± 0.08^{bC}	0.52 ± 0.09^{aB}

The means and SE ($n = 3$) followed by a different lowercase letter within the same column denote a significant difference at $p < 0.05$ among different treatments at each planting period. A different uppercase letter within the same row of each parameter denotes a significant difference at $p < 0.05$ among different planting periods according to Duncan's multiple range test

the plant roots having a retention function of heavy metals (Xia 2004). Our results suggest that cadmium translocation from the root to the shoot was limited when high cadmium content has already accumulated in the shoot, resulting in no significant difference of TF values between the plants inoculated with biochar-immobilized CRB and the uninoculated control. This might be explained that at high cadmium contents, the root cells bind to cadmium, causing cadmium precipitation and stored in vacuoles of the root cortex and in dead cells, resulting in a low translocation rate of cadmium to the shoots via xylem (Shute and Macfie 2006; Zhang et al. 2010).

The highest TF values were found in plants inoculated with BC-*Micrococcus* sp., followed by BC-*Arthrobacter* sp. (0.91 ± 0.07) at 6 weeks after planting. Interestingly, inoculation of BC-*Micrococcus* sp. enhanced a TF from 0.54 ± 0.09 to 0.96 ± 0.08. The explanation would involve the increasing of cadmium solubility by these bacteria (Prapagdee and Wankumpha 2017). The study of Yoon et al. (2006) stated that plants with a TF greater than 1 are considered effective plants for heavy-metal phytoextraction. This concept is also supported by the work of Ali et al. (2013), who reported that a high potential in metal translocation from the roots to shoots is one of the desirable properties for phytoextracting plants. In this case, inoculation of biochar-immobilized CRB enhanced cadmium accumulation capability of *C. laxum* and shifted the TF closer to 1, indicating cadmium phytoextraction. Although one of the limitations of *C. laxum* is a small-sized plant with low biomass, it is easy cultivation in a worldwide geographic growth zone (Wang et al. 2012). Our results point out that inoculation of BC-*Micrococcus* sp. promoted plant growth and inoculation of both biochar-immobilized CRB enhanced cadmium accumulation in the shoot and root of *C. laxum*, providing BAF values higher than 1.0. Plants with PEC or BAF values greater than 1.0 indicate high heavy-metal accumulation (Taiwo et al. 2016). Therefore, *C. laxum* inoculated with biochar-immobilized CRB is suitable to apply for cadmium phytoextraction, in particular in low cadmium-contaminated soil due to a small-sized plant.

Conclusion

Biochar was used for cell immobilization of two cadmium-resistant bacteria, namely *Arthrobacter* sp. and *Micrococcus* sp., and these biochar-immobilized CRB were applied in cadmium-contaminated soil. Biochar-immobilized CRB were able to survive in cadmium-contaminated soil. The inoculation of BC-*Micrococcus* sp. promoted the root dry weight of *C. laxum* planted in cadmium-contaminated soil. Plants inoculated with either BC-*Arthrobacter* sp. or BC-*Micrococcus* sp. had the highest cadmium contents in the shoots, the roots, and a whole plant. Significant increases in PEC, BAF, and TF were found in plants inoculated with either BC-*Arthrobacter* sp. or BC-*Micrococcus* sp. compared to the untreated control. It could be concluded that *C. laxum* combined with BC-*Arthrobacter* sp. or BC-*Micrococcus* sp. inoculation can achieve a high efficiency of cadmium phytoextraction in metal-contaminated soil.

Acknowledgements The authors thank V. Vanitshavit, A. Nisaitrongsrisug, and N. Siribanchachai for their valuable technical assistances. Appreciation is also expressed to Dr. T.N. Stewart for proofreading this article.

Funding information This research was supported by a grant from the Center of Excellence on Biodiversity (grant number BDC-PG1-160003) and the Faculty of Environment and Resource Studies of Mahidol University Alumni Association.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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