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ORIGINAL PAPER

Bacterial-assisted cadmium phytoremediation by *Ocimum gratissimum* L. in polluted agricultural soil: a field trial experiment

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Abstract A field study of cadmium phytoremediation by *Ocimum gratissimum* L. and the potential enhancement by two cadmium-resistant bacteria, *Ralstonia* sp. TISTR 2219 and *Arthrobacter* sp. TISTR 2220, were explored in a cadmium-polluted agricultural area. The results demonstrated the ability of one of the bacterial strains to promote cadmium accumulation in *O. gratissimum* L. planted in soil with cadmium concentrations till 65.2 mg kg^{-1}. After transplantation in contaminated soil for 2 months, soil inoculation with *Arthrobacter* sp. enhanced cadmium accumulation in the roots, above-ground tissues, and whole plant of *O. gratissimum* L. by 1.2-fold, 1.4-fold, and 1.1-fold, respectively, compared with the untreated control. The presence of *Arthrobacter* sp. in soil facilitated cadmium phytoremediation in *O. gratissimum* L. similar to that of an EDTA application. Seeds of *O. gratissimum* L. grown in polluted soil contained undetectable to negligible concentrations of cadmium. Significant increases in the bioconcentration and translocation factors of *O. gratissimum* L. were observed in *Arthrobacter* sp.-inoculated plants at only 2 months post-transplant compared with the uninoculated control. The highest percentage of cadmium removal was found in soil used to cultivate EDTA-treated *O. gratissimum* L., followed by an *Arthrobacter* sp.-inoculated plant. Our findings suggest that the synergistic use of *Arthrobacter* sp. with *O. gratissimum* L., an essential oil-producing crop, could be a feasible economic and environmental option for the reclamation of cadmium-polluted areas.

Keywords Cadmium-contaminated soil · Phytoremediation · African basil · *Arthrobacter* · *Ralstonia*

Introduction

Cadmium is a non-essential metal with the potential to be highly toxic to living organisms. Important sources of cadmium contamination in the environment are zinc mines and smelting plants. Agricultural soil surrounding zinc mining areas in the northern part of Thailand has a high cadmium concentration (Phaenark et al. 2009). Several edible crops in cadmium-contaminated soil can easily take up and accumulate cadmium, thus passing the contamination to consumers via the food chain (Moreno et al. 2002). Excessive intake of cadmium-contaminated food causes Itai–itai disease (Makino et al. 2007). More than 10 % of the villagers who live in this area had higher cadmium concentrations in their blood and urine than what is considered safe (Department of Environmental Quality Promotion 2011). According to the standard of the World Health Organization (WHO), cadmium concentrations in blood and urine that are considered safe levels are no more than 5 μg L^{-1} and 5 μg g^{-1} of creatinine, respectively. The cleanup methods have been urgently developed for the reclamation of this cadmium-polluted agricultural site. Phytoremediation is an alternative green technique that uses plants to remove heavy metals from the environment or to render them harmless by an uptake of contaminants from the root to other parts of the plants (Kumar et al. 1995). In comparison with other remediation technologies, phytoremediation is considered an efficient, low-cost, eco-

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friendly, solar-driven, and socially acceptable technology (Weis and Weis 2004; Ali et al. 2013).

Ocimum gratissimum L., or African basil, is an essential oil-producing or aromatic crop. Essential oil is used as an aromatic agent in several non-food industries as a high-value product (Zheljazkov et al. 2008a). Some aromatic crops can accumulate cadmium, copper, and lead, but these heavy metals do not pass from plant tissues to the extracted essential oils (Zheljazkov et al. 2006). Thus, this plant could be grown safely as a cash crop in cadmium-polluted soil without cadmium contamination in its essential oil. However, there is an important limitation in solely relying on plants for the phytoextraction of metal-contaminated soils, as the degree to which plants are able to take up cadmium also depends on the bioavailability of cadmium in the soil (Benavides et al. 2005). To solve this problem, soil bioaugmentation with bacteria that assists heavy metal phytoremediation is a promising method for cleaning up contaminated soil (Lebeau et al. 2008). Some bacteria are able to increase metal mobility and bioavailability to plants by producing exopolymers (Prapagdee et al. 2012). Exopolymers bind to heavy metals and increase heavy metal mobility in contaminated soil (Jensen-Spaulding et al. 2004). An increase in metal solubility or mobility in the soil leads to better metal uptake by the plant and enhances phytoremediation in contaminated soil.

Current research is lacking in field experimental evidence on the effectiveness of bacterial-assisted cadmium phytoremediation. Our previous research reports that two strains of cadmium-resistant bacteria, *Ralstonia* sp. TISTR 2219 (formerly strain TAK1) and *Arthrobacter* sp. TISTR 2220 (formerly strain TM6), increase the water solubility of cadmium in the soil (Prapagdee et al. 2012). In addition, *Arthrobacter* sp. TISTR 2220 was able to increase cadmium uptake and accumulation in *O. gratissimum* L. in controlled pot experiments (Khonsue et al. 2013). Hence, this is the first experimental study to document the feasibility of using cadmium-resistant bacteria to assist cadmium phytoremediation by aromatic *O. gratissimum* L. crops in a real-world cadmium-polluted agricultural area. This research was performed at a cadmium-contaminated area located in Mae Sot district, Tak Province, northern Thailand, between February 2013 and January 2014.

Materials and methods

Bacterial strains and inocula preparation

Ralstonia sp. TISTR 2219 and *Arthrobacter* sp. TISTR 2220, cadmium-resistant bacteria, were isolated from cadmium-contaminated soil as described by Prapagdee and Watcharamusik (2009) and Prapagdee et al. (2012). These bacteria were cultivated in Luria–Bertani (LB) broth (Criterion, USA) at 30 °C with 150 rpm shaking. Bacterial inocula were prepared according to the methods described by Prapagdee et al. (2013). The cell turbidity of the prepared bacterial cells was adjusted to an optical density of 0.2 at 600 nm (OD_{600}), and the viable cell numbers of each bacterial strain were approximately 2×10^{10} CFU mL^{-1}.

Cultivar of *O. gratissimum* L.

Ocimum gratissimum L. seedlings were grown in 25 cm diameter of plastic pots containing garden soil for 3 months under greenhouse conditions. The stem height of 3-month-old *O. gratissimum* L. plants was approximately 20 cm. The plants were transplanted into cadmium-contaminated soil at the study site.

Description of the experimental study site

This study site is located near zinc mines and smelting plants in Mae Sot district, Tak Province, northern Thailand (N 16° 40.593, E 098° 37.630) (Fig. 1). The main use of the land at this site is to grow edible crops such as rice, maize, mung bean, and soybean. Cadmium contamination in this area has been detected in soil and crops since 2000. Polluted agricultural soil containing cadmium at a concentration of 65.2 ± 2.2 mg kg^{-1} was selected for this study. The concentration of cadmium bioavailability (DTPA-extractable concentration) was 26.13 ± 0.45 mg kg^{-1}. The physical and chemical characteristics of soil at this site were as follows: loamy soil texture, pH (1:1 w/v H_2O) 7.2, electrical conductivity (1:5) of 0.20 mS cm^{-1}, cation exchange capacity of 13.8 cmol kg^{-1}, 3.8 % organic matter, 0.19 % total nitrogen, 10.7 mg kg^{-1} available phosphorous, 80 mg kg^{-1} extractable potassium, 363 mg kg^{-1} extractable sodium, 5155 mg kg^{-1} extractable calcium, and 555 mg kg^{-1} extractable magnesium (Prapagdee et al. 2013). The bulk density of this agricultural soil was 1.7 g cm^{-3}. The number of indigenous, viable soil bacteria in the contaminated soil was determined by spreading onto LB agar supplemented with 3 mM cadmium nitrate ($Cd(NO_3)_2$), and the number of indigenous viable soil bacteria was 1.4×10^3 CFU g^{-1} (3.2 $\log_{10}$ CFU g^{-1}).

Field trial experiments

Preparation of plots for plant cultivation and experimental design

The experimental plot was 30 m^2 with a width of 5 m and a length of 6 m. The soil was ridged at a width of 50 cm, a length of 6 m, and a height of 15 cm for four rows. Each

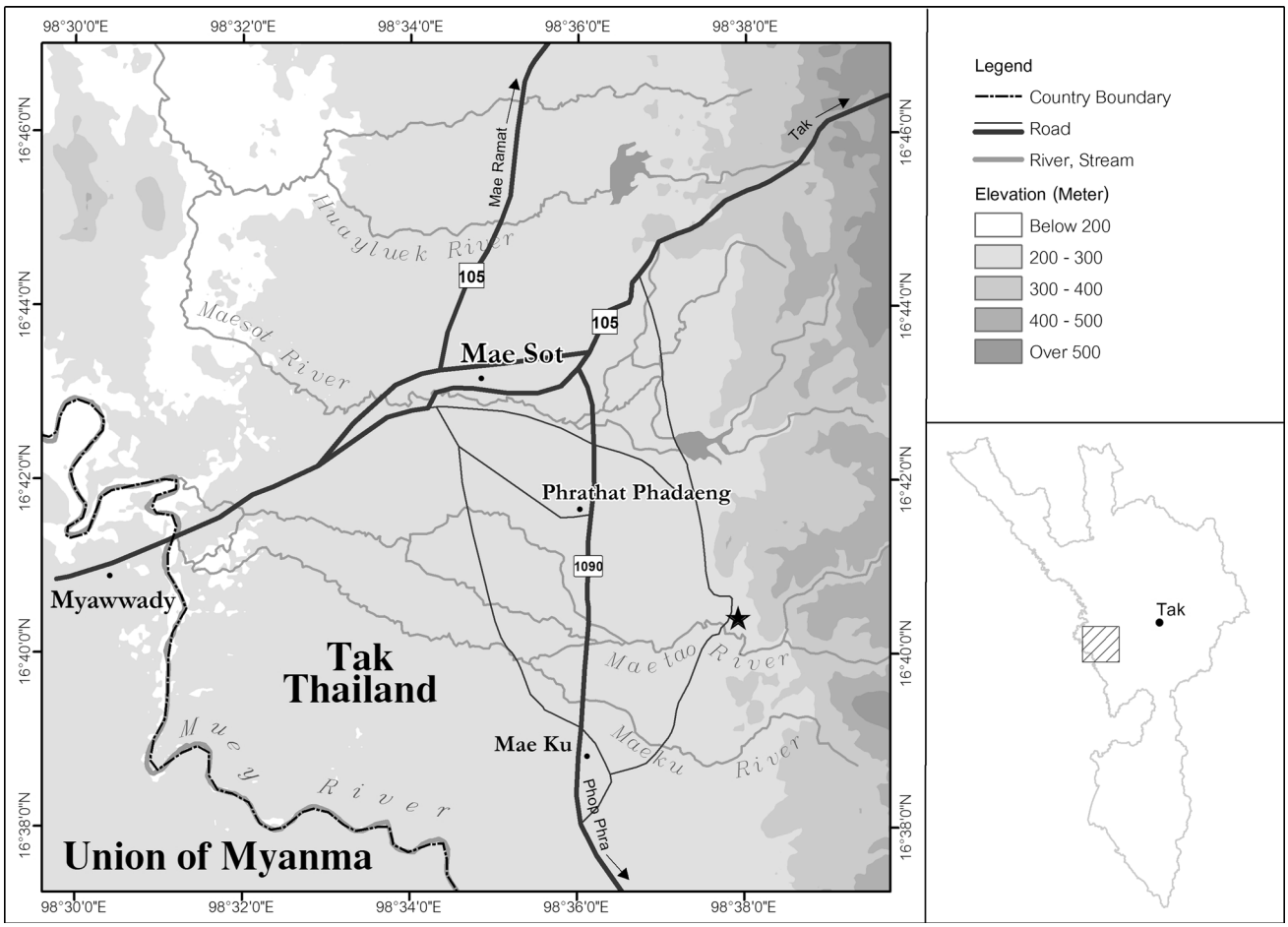

Fig. 1 Location map of cadmium-polluted site (*star symbol*) at Mae Sot District, Tak Province, northern Thailand

row was divided into two sub-rows and set for one treatment. There were a total of four treatments with eight sub-rows. Eight of 3-month-old *O. gratissimum* plants were transplanted into each sub-row with a 35-cm interval between each plant. The first row was a control treatment with no bacterial inoculation. The second and third rows were inoculated with *Ralstonia* sp. TISTR 2219 and *Arthrobacter* sp. TISTR 2220, respectively. The size of the bacterial inoculum applied to the soil of each treatment was 1 % (v/w). A suspension of each bacterial inoculum was directly sprayed in the soil at the area of the plant root zone and mixed thoroughly before plantation. A synthetic chelating agent, ethylenediaminetetraacetic acid (EDTA), was added to the fourth row at a concentration of 30 mg kg^{-1} as a positive chemical control. The total volume of bacterial inocula and EDTA added to the soil was calculated based on the bulk density of soil at 20 cm depth based on the plant root. Plants were watered daily and sprayed with insecticide weekly for 6 months. The water pH for irrigation was 7.85. Cadmium concentration in water used in this experiment was below 0.01 mg L^{-1}, the detection limit of a flame atomic adsorption spectrophotometer (FAAS; Varian spectra model AA240FS, USA).

Plant and soil analysis

At least three plant samples were randomly selected from each sub-row at 2 and 6 months after transplantation. Rhizosphere soil was aseptically collected, and the number of viable rhizobacteria was determined by spreading on LB agar containing 3 mM $Cd(NO_3)_2$. Harvested plants were thoroughly washed with tap water and rinsed twice with deionized water. The plants were divided into root and shoot parts and oven-dried at 80 °C before being weighed. Plant growth was evaluated by measuring the stem height, root length, and dry weight. Cadmium concentration in each part of the dried plants was acidic digested in 2:1 of HNO_3 and $HClO_4$ by volume with the microwave digestion method (Milestone model Ethos One, Italy) according to the methods of US EPA method 3052 and Simmons et al. (2003). Cadmium concentrations in digested samples of soil and plants were analyzed by a FAAS. Soil samples

were also collected at 2 and 6 months. Each soil sample was digested in a mixture of concentrated HNO_3 and concentrated HCl (1:3 by volume) with the microwave digestion method (McGrath and Cunliffe 1985). Cadmium concentration in acid-digested soil was measured by FAAS.

Data analysis

The ratio of dry weight roots to dry weight shoots was calculated for comparison of plant growth in each treatment (Chiu et al. 2006). The bioconcentration factor (BCF) was determined by calculating the ratio of metal concentration in the plant to that in the soil around the plant roots (Kumar et al. 1995). The translocation factor (TF) was calculated by dividing metal concentration in the above-ground tissues with that in the roots (Mattina et al. 2003). The total cadmium accumulation in plants was calculated by multiplying cadmium concentration in plant tissues with dry weight biomass.

Statistical analysis

Each experiment was performed at least in triplicate. The mean ($\bar{x}$) and standard error (SE) of plant growth and cadmium concentration in plants and soil for all treatment groups were calculated. We report the results based on the one-way analysis of variance followed by the Duncan multiple range test at 95 % confidence intervals.

Results and discussion

Effects of cadmium-resistant bacteria on the growth performance of *O. gratissimum* L. transplanted into cadmium-polluted soil

We conducted this study over a period of 6 months. The results showed that all *O. gratissimum* L. plants grew normally in the heavily cadmium-contaminated soil (65.2 ± 2.2 mg kg^{-1}) without suffering from phytotoxicity. There was no sign of growth retardation during plantation in cadmium-contaminated soil. Main visible symptoms of cadmium toxicity such as chlorosis, leaf rolls, and stunting, were not observed in *O. gratissimum* L. The results of the plant growth and biomass measurements are presented in Table 1. The root length, shoot height, and total dry biomass of *O. gratissimum* L. did not significantly increase ($p < 0.05$) after the bacterial or EDTA applications compared with the control group during both the 2- and 6-month periods. Both growth rate and plant yield of *O. gratissimum* L. are high under these experimental conditions. The dry plant biomass at 6 months after transplant in all treatments increased by 6.5-fold–8.1-fold in comparison with plants at 2 months after transplant.

In addition, the root-to-shoot ratios of plants inoculated with *Ralstonia* sp., *Arthrobacter* sp., and EDTA were not significantly different from the control group. Higher shoot dry weights were observed for all treatments at 6 months after transplant, resulting in a lower root-to-shoot ratio compared with the ratios at 2 months post-transplant. Previous studies have been shown that the root-to-shoot ratio is high in stressful environments and nutrient-deficient conditions, and shoot tissues are more sensitive to heavy metal toxicity than are root tissues (Chiu et al. 2006). In general, cadmium inhibits root and shoot lengths as well as overall plant growth and interferes with nutrient uptake (Belimov et al. 2005; Benavides et al. 2005). However, some plants have evolved mechanisms to control the uptake, accumulation, and detoxification of heavy metals, including the use of phytochelatins and metallothioneins (Benavides et al. 2005). Our findings indicated that *O. gratissimum* L. tolerated cadmium toxicity in soil at a cadmium concentration till 65.2 mg kg^{-1}.

These results revealed that none of the tested bacterial strains directly promoted plant growth. It was due to *Ralstonia* sp. and *Arthrobacter* sp. that produced very low levels of a plant growth hormone, indole-3-acetic acid (IAA) (Khonsue et al. 2013). In contrast, plant-growth-promoting rhizobacteria (PGPR) can promote plant growth when cultivated in heavy metal-contaminated soil. Copper-resistant *Achromobacter xylosoxidans* Ax10, which produced IAA and solubilized phosphate, can promote an increase in the root length, shoot length, fresh weight, and dry weight of Indian mustard (*Brassica juncea*) (Ma et al. 2009). Cadmium-resistant PGPR promoted the growth of rape (*Brassica napus*) (Sheng and Xia 2006; Dell' Amico et al. 2008) and sunflower (*Helianthus annuus*) (Prapagdee et al. 2013). However, some PGPR facilitated the growth of one plant but failed to promote the growth of others. The heavy metal-resistant strain *Burkholderia* sp. J62 promoted the growth of maize (*Zea mays*) and tomato (*Lycopersicon esculentum*) but was not able to promote the growth of *B. juncea* due to its ability to colonize the root of its host plant (Jiang et al. 2008).

Enhanced cadmium bioaccumulation in *O. gratissimum* L. after soil inoculation with cadmium-resistant bacterium

The ability of these bacteria to enhance cadmium phytoextraction by *O. gratissimum* L. planted in the field investigations is shown in Table 2. *O. gratissimum* L. grown in cadmium-contaminated soil can accumulate cadmium in the roots and above-ground tissues. At 2 months post-

Table 1 Growth performances of *O. gratissimum* L. planted in cadmium-polluted soil for 2 and 6 months

Treatment	Root length (cm)		Stem height (cm)		Dry weight of whole plant (g)		Root:shoot ratio	
	Month 2	Month 6	Month 2	Month 6	Month 2	Month 6	Month 2	Month 6
No bacterial inoculation	21.0 ± 1.6	27.7 ± 0.6	65.7 ± 8.1	157.0 ± 11.2	61.3 ± 5.7	460.3 ± 25.2	0.24 ± 0.03	0.10 ± 0.06
Ralstonia sp.	24.0 ± 1.9	28.3 ± 7.1	71.0 ± 4.6	173.7 ± 14.0	67.6 ± 6.8	441.7 ± 38.4	0.21 ± 0.05	0.10 ± 0.05
Arthrobacter sp.	23.3 ± 1.7	24.7 ± 7.5	66.3 ± 7.5	182.3 ± 14.6	59.5 ± 8.2	485.8 ± 28.0	0.21 ± 0.03	0.09 ± 0.04
EDTA	21.7 ± 2.9	28.3 ± 4.7	62.2 ± 6.4	165.1 ± 12.4	62.3 ± 2.8	476.1 ± 30.5	0.19 ± 0.04	0.08 ± 0.04

The means and the SE ($n = 3$) were not significantly different ($p < 0.05$) according to the one-way analysis of variance

Table 2 Cadmium accumulation in each part of *O. gratissimum* L. planted in cadmium-polluted soil for 2 and 6 months

Treatment	Cadmium content (μg g^{-1} plant dry weight)						Total cadmium accumulation (mg plant $tissue^{-1}$)	
	Root		Above-ground tissue		Whole plant			
	Month 2	Month 6	Month 2	Month 6	Month 2	Month 6	Month 2	Month 6
No bacterial inoculation	8.55 ± 0.43[a]	12.35 ± 0.63[a]	4.05 ± 0.28[a]	2.81 ± 0.48[a]	5.40 ± 0.24[a]	4.14 ± 0.21[a]	0.30 ± 0.02[a]	1.81 ± 0.32[a]
Ralstonia sp.	9.40 ± 0.57[a]	11.85 ± 0.84[a]	4.28 ± 0.50[a]	2.99 ± 0.37[a]	5.39 ± 0.10[a]	4.50 ± 0.37[a]	0.31 ± 0.03[a]	2.08 ± 0.28[a]
Arthrobacter sp.	10.53 ± 0.25[b]	11.60 ± 0.42[a]	5.80 ± 0.34[b]	3.23 ± 0.34[a]	6.07 ± 0.34[b]	4.02 ± 0.39[a]	0.41 ± 0.04[b]	2.01 ± 0.19[a]
EDTA	10.80 ± 0.37[b]	14.10 ± 0.29[b]	5.57 ± 0.55[b]	3.70 ± 0.61[a]	5.95 ± 0.28[b]	5.37 ± 0.35[b]	0.39 ± 0.02[b]	2.16 ± 0.49[a]

The means and the SE ($n = 3$) followed by the same lowercase letter within column were not significantly different ($p < 0.05$) according to Duncan's multiple range test

transplant, the presence of *Arthrobacter* sp. in cadmium-contaminated soil significantly enhanced cadmium contents in the roots, above-ground tissues, and whole plant of *O. gratissimum* L. by 1.2-fold, 1.4-fold, and 1.1-fold, respectively, compared with the untreated control (Table 2). The synergistic effect of *Arthrobacter* sp. on promoting cadmium accumulation in *O. gratissimum* L. was similar to that of an EDTA application. However, cadmium accumulation in each plant part and in the whole plant of *O. gratissimum* L. inoculated with *Ralstonia* sp. was not significantly different ($p \geq 0.05$) compared with the uninoculated control.

These results highlighted the fact that soil inoculation with *Arthrobacter* sp. was an effective approach to enhance the cadmium accumulation in *O. gratissimum* L. at 2 months after transplant. Other studies also showed that cadmium-resistant bacteria can enhance cadmium uptake by plants. Specifically, the cadmium-resistant bacteria *Pseudomonas* sp. RJ10 and *Bacillus* sp. RJ16 increased cadmium uptake in cadmium-hyperaccumulating tomatoes (*L. esculentum*) (He et al. 2009). Cadmium-resistant PGPR promoted cadmium accumulation in *H. annuus* (Prapagdee et al. 2013). The co-application of citric acid and metal-resistant microorganisms increased cadmium accumulation by 25–35 % in black nightshade (*Solanum nigrum*), a cadmium hyperaccumulator (Gao et al. 2012).

Unfortunately, there was no increase in the cadmium uptake of *O. gratissimum* following soil inoculation with tested bacteria at 6 months after transplant, except EDTA application (Table 2). Cadmium accumulation in the above-ground tissues at 6 months after transplant was lower than that at 2 months, indicating less cadmium translocation from roots to shoots. There are highly uptake nutrients and other metal ions during the blossom and seed-filling stages. In general, plants uptake metal ions from soil either by passive transport with the mass flow of water into the roots or by active transport whereby the contaminants cross the plasma membrane of root epidermal cells (Kim et al. 2003). Further evidence to this conclusion is that decreasing numbers of viable rhizobacteria were present in the soil over time (Fig. 2). In addition, the survivability of *Ralstonia* sp. and *Arthrobacter* sp. in the sterile cadmium-contaminated soil before applying them in the pot experiment was monitored. The results found that the number of *Ralstonia* sp. in the sterile cadmium-contaminated soil was changed from 6.7×10^7 on the first day to 3.7×10^5 CFU g^{-1} at 10 days after incubation. The number of *Arthrobacter* sp. in the sterile cadmium-contaminated soil from the first day to the tenth day was from 8.7×10^7 to 5.5×10^5 CFU g^{-1}. The highest numbers of viable cells of *Ralstonia* sp. and *Arthrobacter* sp. in the sterile cadmium-contaminated soil were 1.3×10^{10} and

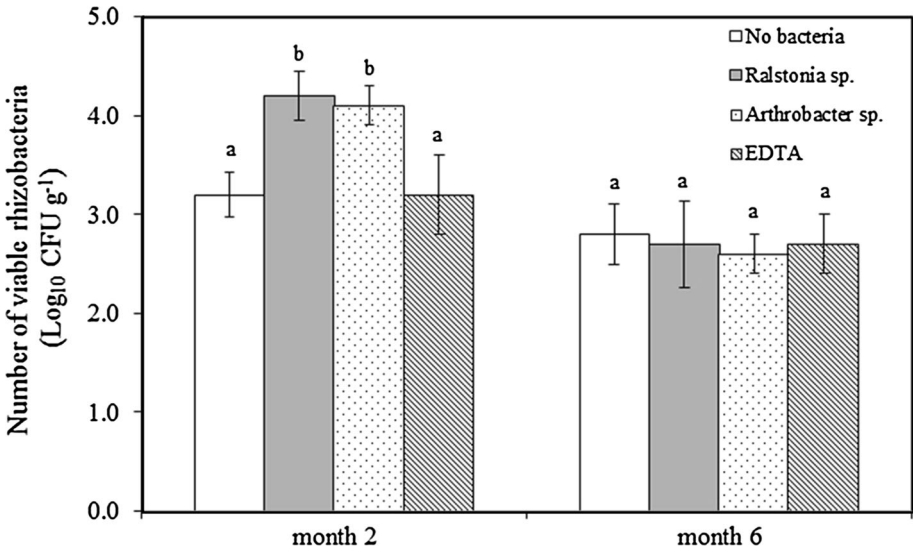

Fig. 2 Number of viable rhizobacteria in the rhizosphere soil of *O. gratissimum* L. in the treatments that were inoculated with *Ralstonia* sp. or *Arthrobacter* sp. and supplemented with EDTA compared with the uninoculated control at 2 and 6 months after transplant in cadmium-polluted soil. The *error bars* represent the SE ($n = 3$), and the *lowercase letter above the bar graph* denotes a significant difference ($p < 0.05$) compared with the control treatment

8.3×10^9 CFU g^{-1} at the fourth day of incubation, respectively. These results suggested that periodically repeated soil inoculation with *Arthrobacter* sp. every week is required to prolong the cadmium phytoextraction efficiency. In addition, soil inoculation with immobilized bacterial cells offers enormous advantages in cell protection and survival in contaminated soil.

The cadmium contents in the roots and in whole *O. gratissimum* L. plants treated with EDTA were higher than those that were inoculated with cadmium-resistant bacteria, increased by 1.1-fold and 1.3-fold, respectively, compared with the uninoculated control. The bioavailability of heavy metals is an indicator of the plant's ability to accumulate heavy metals from the soil (Branquinho et al. 2007). EDTA increases the solubility and bioavailability of heavy metals in soil, resulting in increased plant uptake of heavy metals (Evangelou et al. 2007). Our finding is consistent with other studies. The application of EDTA in soil increased the cadmium solubility, and the cadmium concentration in the shoots of *Z. mays* and white bean (*Phaseolus vulgaris*) was reported by Luo et al. (2005). Prapagdee et al. (2013) show that cadmium accumulation in the whole *H. annuus* treated with EDTA was higher than that of *H. annuus* inoculated with cadmium-resistant PGPR.

In contrast, there was no significant difference ($p < 0.05$) in the cadmium contents of the above-ground tissues from *O. gratissimum* L. in all treatments after 6 months of cultivation (Table 2). On comparing the total cadmium accumulation in *O. gratissimum* L. at 2 and 6 months post-transplant, the total cadmium accumulation in each plant (mg plant $tissue^{-1}$) for all treatments was higher at the 6-month harvest than at the 2-month harvest by approximately fivefold–sixfold due to a significant increase in plant biomass during the 6-month harvest period (Table 1). Interestingly, *O. gratissimum* L. seeds had very low to undetectable cadmium concentrations. The maximum cadmium content (0.03 μg g^{-1}) was detectable only in the seeds from *O. gratissimum* L. plants treated with EDTA. Cadmium contents in the seeds of other treatments were lower than 0.01 μg g^{-1}. The cadmium accumulation in each plant part decreased in the order of root > stem > leaf > fruit > seed (Benavides et al. 2005). In Japan, the paddy fields that have produced rice grains containing cadmium at concentrations >1 μg g^{-1} are designated as contaminated paddy fields (Makino et al. 2007). Zheljazkov et al. (2006) found that there was no detectable cadmium, copper, or lead contents in the oils of dill (*Anethum graveolens*), peppermint (*Mentha* × *piperita*), or basil (*Ocimum basilicum*) that were grown in soils amended with these heavy metals with no alterations of oil composition. There was also no risk of transferring heavy metals to the oils of these crops. Our results suggested that *O. gratissimum* L. could be grown as an alternative to edible plants in cadmium-polluted agricultural soil. The improvement in the interactions between plants and cadmium-resistant bacteria for the enhancement of cadmium uptake by aromatic crops is considered an important strategy for cadmium phytoextraction in polluted soil.

Cadmium-resistant bacterium facilitates cadmium accumulation and translocation in *O. gratissimum* L.

The results in Fig. 3a show that the BCF of *O. gratissimum* L. treated with *Arthrobacter* sp. and EDTA at 2 months post-transplant was higher than that of the untreated control. However, no increase was observed in the BCF of *O. gratissimum* L. at 6 months after transplant with *Arthrobacter* sp. This result concurred with the observation

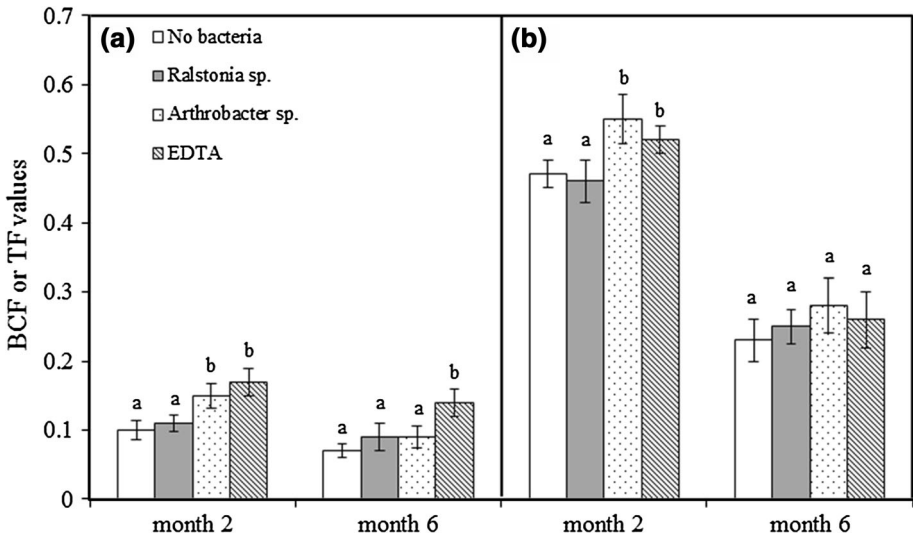

Fig. 3 Bioconcentration (**a**) and translocation (**b**) factors of cadmium in *O. gratissimum* L. planted in cadmium-polluted soil inoculated with *Ralstonia* sp. or *Arthrobacter* sp. and supplemented with EDTA compared with the uninoculated control for 2 and 6 months. The *error bars* represent the SE ($n = 3$), and the *lowercase letter above the bar graph* denotes a significant difference ($p < 0.05$) compared with the control treatment

of cadmium accumulation in each plant part as well as with whole *O. gratissimum* L. inoculated with *Arthrobacter* sp. during the 6 months of growth (Table 2). *O. gratissimum* L. had a low BCF in both harvested periods. The BCFs observed in this field study were slightly low due to the high cadmium concentration and low cadmium availability in the soil. The bioavailability factor in some medicinal plants planted in heavy metal-contaminated soil increased with decreasing cadmium concentrations in the soil (Zheljazkov et al. 2008b). The causative factor leading to a low BCF might be due to cadmium competition with other metals in the soil. During uptake, cadmium ions compete for the same transmembrane carrier used by other metal ions such as zinc, copper, iron, manganese, and nickel (Benavides et al. 2005). Agricultural soil in this study area contained not only cadmium but also high concentrations of zinc (826.0 mg kg^{-1}). Accumulated metals in plant roots and their translocation into plant tissues decrease in the order of zinc > cadmium > lead (Boruvka et al. 1997).

The TFs of *O. gratissimum* L. at 2 months after transplant were higher than those of *O. gratissimum* L. at 6 months for all treatments (Fig. 3b). The highest TF was found in plants inoculated with *Arthrobacter* sp. during the 2-month growth period; however, there was no significant difference ($p \geq 0.05$) between this group and the EDTA-treated *O. gratissimum*. In the 6-month growth period, the TFs of all *O. gratissimum* L. treatment groups were not significantly different ($p < 0.05$). The TFs of *O. gratissimum* L. were still quite low in comparison with other cadmium-hyperaccumulating plants (Sun et al. 2008; Phaenark et al. 2009). Heavy metal phytoextraction required the translocation of heavy metals to the easily harvestable plant parts (Kim et al. 2003). EDTA in the soil stimulated cadmium translocation from the roots to the shoots of *B. juncea* by changing the cadmium solubility and bioavailability of cadmium in the soil (Jiang et al. 2003). Similar results noting increased cadmium translocation from the roots to the shoots in other plants after soil inoculation with cadmium-resistant bacteria were reported in other studies (Prapagdee et al. 2013; Khonsue et al. 2013). In general, cadmium is accumulated primarily in the root tissue; only small amounts of cadmium are translocated to the shoot tissue (Benavides et al. 2005). A higher degree of cadmium accumulation in the roots versus in the shoots led to less translocation of cadmium from the root to the shoot system. According to this study, the cadmium content in the roots was higher than that of above-ground tissues for both harvest periods (Table 2). Zheljazkov et al. (2008b) reported that cadmium, lead, and copper accumulated primarily in the roots, whereas higher concentrations of manganese and zinc were found in the leaves of several medicinal plants.

The efficiency of cadmium phytoremediation is also dependent on the cadmium removal from contaminated soil. Two and six months after transplant, the cadmium concentrations in contaminated soil were decreased for all treatment groups. The percentages of cadmium removal from the soil planted with *O. gratissimum* L. alone or treated with *Ralstonia* sp., *Arthrobacter* sp., and EDTA during the 2-month harvest periods were 18.9, 20.4, 25.4, and 31.3 %, respectively. The amount of cadmium that was removed by *O. gratissimum* L. inoculated with *Arthrobacter* sp. and EDTA was significantly higher than that removed by *O. gratissimum* L. inoculated with *Ralstonia* sp. and the uninoculated control. The amounts of cadmium in the soil after transplant with *O. gratissimum* L. for 6 months in all treatments, including the plants uninoculated and inoculated with *Ralstonia* sp., *Arthrobacter* sp., and EDTA, were decreased by 17.0, 18.1, 20.2, and 22.4 %, respectively. The percentages of soil

cadmium removal were less than those observed 2 months after transplant. Plants had a lower root-to-shoot ratio at 6 months than that at 2 months, because this growth stage is the declining stage. As previously indicated, cadmium highly accumulated in root tissue. Plants exhibit no significant release of cadmium after cadmium exposure; however, wetland plants may excrete the metals through salt glands (Hardy and O'Keeffe 1985; Weis and Weis 2004). However, it was a possibility that dead root tissue was decomposed by indigenous microorganisms and returned cadmium to the soil environment. Our results confirmed the results of other reports of the ability of EDTA to enhance the phytoremediation of heavy metals (Jiang et al. 2003; Luo et al. 2005). The results of this study clearly indicate that the ability of *Arthrobacter* sp. to improve cadmium phytoremediation by *O. gratissimum* L. was nearly as efficient as that of EDTA application. EDTA is hardly to degrade in the environment, and it can be toxic to plants and soil microorganisms (Luo et al. 2006). Thus, soil inoculation with *Arthrobacter* sp. is much more environmentally sound compared with EDTA application. The role of *Arthrobacter* sp. increased soil cadmium solubility and is related to the production of exopolysaccharide (EPS) (Prapagdee et al. 2012). EPS forms complexes with metal cations by electrostatic interaction and promotes metal plant uptake (Chen et al. 1995; Pal and Paul 2008). Therefore, the cultivation of aromatic *O. gratissimum* L. plants coupled with *Arthrobacter* sp. as a co-bioremediator in contaminated agricultural soil is a feasible option for remediating cadmium-polluted soil.

To study mass balance of cadmium in soil and the whole plant, the volume of soil was estimated based on the bulk density of soil and the area of plant root boundary at 20 × 20 × 20 cm ($W \times L \times D$). The sum of cadmium accumulated in the whole plant and cadmium that remained in the soil after plantation was less than the initial cadmium concentration in the soil (Table 3). The loss of cadmium after transplantation was calculated in terms of percentage. The highest percentage of cadmium loss was found in treatment with EDTA application at both harvested periods. EDTA can increase the metal solubility and promote the leaching of soil metals (Jiang et al. 2003; Evangelou et al. 2007). The loss of soil cadmium in treatment with EDTA application would involve the leaching to underground water. However, the effect of leaching during watering of plants might be low, because the water for irrigation is slightly alkaline (pH 7.84) and cadmium is more soluble and leaching in acid condition. Our explanation is confirmed by the study by Ok et al. (2004) which reported that water-soluble fraction of cadmium in contaminated soil is very low. The main fractions of soil cadmium are exchangeable and acid-digested fractions. Moreover, the high organic matter contents showed the low water-soluble cadmium concentration in soil (Crommentuijn et al. 1997). Our tested soil had a slightly high% organic matter (3.8 %); therefore, the leaching of soil cadmium should be low. In addition, the percentages of cadmium loss in soil inoculation with *Ralstonia* sp. and *Arthrobacter* sp. were higher than those of the uninoculated control. These results corresponded well to the study of Khonsue et al. (2013), who claimed that *Ralstonia* sp. and *Arthrobacter* sp. promote cadmium solubility in soil.

Because of the low rate of cadmium removal from contaminated soil, more time to repeat cultivation would be required to remediate a polluted site. The time required for remediation ranges from 1 to 20 years depending on the type and concentration of heavy metals, the heavy metal removal efficiency of plants, and the plant growth rate (Padmavathiamma and Li 2007). In general, the use of heavy metal-hyperaccumulating plants has been recommended for phytoextraction of soil contaminated with heavy metals (Wang et al. 2008). *O. gratissimum* L. is not a heavy metal-hyperaccumulating plant; however, it could be grown as a high-value aromatic crop in contaminated agricultural soil without cadmium contamination in its oil products. Thus, cadmium phytostabilization using *O. gratissimum* L. would have been a better option for phytoremediation. Several aromatic and medicinal crops may

Table 3 Sum of cadmium content in soil ($n = 3$) and the whole plant ($n = 3$) before and after transplantation for 2 and 6 months

Treatment	Month 2		Month 6	
	Cadmium content (mg)	Cadmium loss (%)	Cadmium content (mg)	Cadmium loss (%)
Before transplantation (only in soil)	886.7	–	886.7	–
After transplantation				
No bacterial inoculation	718.4	19.0	781.5	11.9
Ralstonia sp.	670.9	24.3	720.0	18.8
Arthrobacter sp.	688.1	22.4	736.3	17.0
EDTA	609.2	31.3	707.6	20.2

offer an alternative phytoremediation option for mild-to-moderate contaminated soils without contamination of their marketable products (Zheljazkov et al. 2008a, b). In fact, soils are often contaminated with multiple heavy metals. Therefore, the performance of *O. gratissimum* L. for phytoremediation of other heavy metals should be further evaluated. In addition, we suggest that a combined bioaugmentation with *Arthrobacter* sp. and PGPR strain in contaminated soil would be suitable for stimulating cadmium uptake and plant growth.

Conclusion

Ralstonia sp. and *Arthrobacter* sp. did not promote the growth of *O. gratissimum* L. The root-to-shoot ratio of *O. gratissimum* L. at 2 months after transplantation was higher than that at 6 months. Soil inoculation of an *Arthrobacter* sp. can enhance cadmium accumulation and translocation of cadmium from the roots to the shoots of *O. gratissimum* L. during a 2-month harvest period. However, no increase in the cadmium accumulation and translocation in *O. gratissimum* L. was observed at 6 months after transplantation in contaminated soil. Numbers of viable rhizobacteria in rhizosphere soil at 6 months after transplant decreased with time. Repeated soil inoculation with the *Arthrobacter* sp. would be required for the continuous stimulation of phytoremediation efficiency. Seeds of *O. gratissimum* L. contained undetectable to negligible concentrations of cadmium. Soil with such a high level of cadmium contamination should be used only for the cultivation of aromatic crop plants. This work was to explore the synergistic ability of plants and cadmium-resistant bacteria to enhance the cadmium phytoremediation efficiency in a cadmium-polluted field site.

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