Effects of Cadmium Resistant Bacteria on Promoting Cadmium Phytoremediation in Cadmium contaminated Soil by *Chlorophytum* spp.

ประสิทธิภาพของแบคทีเรียต้านทานแคดเมียมในการส่งเสริมการบำบัดดินปนเปื้อนแคดเมียมโดยใช้ว่านเศรษฐี

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ABSTRACT

This research aimed to study the effects of two strains of cadmium resistant bacteria, namely *Micrococcus* sp. MU1 and *Arthrobacter* sp. TM6, on promoting plant growth and increasing cadmium phytoremediation by two species of *Chlorophytum* spp., namely, *C. comosum* and *C. amaniense*. The pot experiments of each plant were divided into 4 treatments, includings 1) uninoculated control, 2) inoculated with *Micrococcus* sp., 3) inoculated with *Arthrobacter* sp., and 4) inoculated with both bacteria. All plants were tansplanted in cadmium contaminated soil collected from Mae Sot Distract, Tak Province. The results showed that *Micrococcus* sp. significantly promoted the highest root length and dry biomass of *C. comosum* and *C. amaniense* compared to those of other treatments. Cadmium was more accumulated in the roots than in the shoots of both plants. *C. comosum* accumulated cadmium higher than *C. amaniense*. In addition, the highest cadmium contents in the roots and shoots of both plants were observed in plants which were inoculated with *Arthrobacter* sp. Our findings indicate the synergistic interactions between cadmium resistant bacteria and *Chlorophytum* spp. on promoting phytoremediation efficiency in cadmium contaminated soil.

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของแบคทีเรียต้านทานแคดเมียม 2 สายพันธุ์ คือ *Micrococcus* sp. MU1 และ *Arthrobacter* sp. TM6 ในการส่งเสริมการเจริญเติบโตของพืชและช่วยเพิ่มประสิทธิภาพการบำบัดแคดเมียมของพืชไม้ประดับ 2 ชนิด ได้แก่ เศรษฐีเรือนในและเศรษฐีก้านทอง โดยการศึกษาวิจัยนี้แบ่งพืชแต่ละชนิดเป็น 4 ชุดการทดลองคือ 1) ชุดควบคุม 2) ชุดการทดลองที่เติม *Micrococcus* sp. 3) ชุดการทดลองที่เติม *Arthrobacter* sp. และ 4) ชุดการทดลองที่เติมแบคทีเรียทั้งสองชนิด โดยปลูกพืชในดินปนเปื้อนแคดเมียมที่เก็บจากพื้นที่ปนเปื้อนใน อ.แม่สอด จ.ตาก ผลการศึกษาพบว่าชุดการทดลองที่มีการเติมแบคทีเรียต้านทานแคดเมียม *Micrococcus* sp. MU1 ช่วยส่งเสริมให้พืชทั้งสองชนิดมีความยาวรากและมวลชีวภาพสูงมากกว่าชุดการทดลองอื่นๆ และพบว่าแคดเมียมมีการสะสมในส่วนรากมากกว่าส่วนยอดของพืชทั้งสองชนิดโดยเศรษฐีเรือนใน มีการสะสมแคดเมียมสูงกว่าเศรษฐีก้านทอง นอกจากนี้ยังพบว่าพืชทั้งสองชนิดในชุดการทดลองที่มีการเติม *Arthrobacter* sp. TM6 มีการสะสมแคดเมียมในส่วนรากและยอดสูงสุด จากผลการศึกษานี้แสดงให้เห็นถึงการทำงานร่วมกันระหว่างแบคทีเรียต้านทานแคดเมียมและพืชตระกูลว่านเศรษฐีในการส่งเสริมประสิทธิภาพของพืชในการบำบัดดินปนเปื้อนแคดเมียม

Keywords: Cadmium resistant bacteria, Cadmium Phytoremediation, *Chlorophytum* spp.
คำสำคัญ: แบคทีเรียต้านทานแคดเมียม ว่านเศรษฐี การบำบัดแคดเมียมโดยพืช

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Introduction

Soil is a natural resource that is vital to human life, but soil becomes the major sink for heavy metals released into the environment by the emission from several anthropogenic activities. In particular, cadmium is a highly toxic heavy metal. It is one of the important heavy metals contaminated in the soil because cadmium and its compounds may transfer through soil (ATSDR, 2012). In Thailand, cadmium contamination in the agricultural soils has been found in rice fields in Mae Sot District, Tak Province, Thailand (Department of Pollution Control, 2004). Therefore, cadmium contamination in soil must be remediated and an appropriate treatment process is urgently developed. Phytoremediation is a green technology that reclaims contamination soil with heavy metal. One method of phytoremediation is phytoextraction which involves the use of living plant to remove toxic of heavy metals from contaminated soil by uptake of heavy metals into the roots and accumulation in plant tissues (Kumar *et al.,* 1995). However, the efficiency of phytoextraction on the removal heavy metals from contaminated soil depends not only on the plant species but also on the functions of rhizobacteria (Prapagdee *et al.,* 2013).

Thus, this research used two strains of cadmium resistant bacteria, namely *Micrococcus* sp. MU1 that the research of Prapagdee *et al*. (2013) has been reported *Micrococcus* sp. MU1 that can be producing indole-3-acetic acid (IAA) during the late stationary phase of growth that IAA is able to stimulate root elongation, promoted plant growth, and cadmium uptake in contaminated soil by phytoextraction in sunflowers (*Helianthus annuus*) and *Arthrobacter* sp. TM6 was interested in applied to bioremediation by phytoremediation associate with bioaugmentation that is able to increases metal mobility and bioavailability to plant, also there are increased cadmium solubility in soil contaminated of cadmium.Moreover, *Arthrobacter* sp. TM6 can increase cadmium uptake and accumulation in *O. gratissimum* L. according to Khonsue *et al*. (2013) reported.

The ornamental plants are popular and become an appropriate plant of phytoremediation (Liu *et al.,* 2015). One of ornamental plants in the genus *Chlorophytum* has some practical applications in the reclamation of heavy metal polluted areas. Several researches are representing the ability of *Chlorophytum comosum* to remedial pollutants in the environmental specifically the phytoextraction of heavy metal (i.e. cadmium, lead, mercury) contaminated in water or soil. The study of Wang *et al.* (2012) reported that *C. comosum* had a high tolerance to cadmium toxicity and low concentration of cadmium can be stimulated the growth of this plant. Also, it can be said that *C. comosum* can not only valuable economic benefits as ornamental plants but also be used as a plant species for phytoremediation. *Chlorophytum amaniense* is a member of the family Liliaceae and belongs to the same genus of *C. comosum* but there are no any investigations of cadmium phytoremediation by *C. amaniense*. In addition, lack of the study of the enhancing of cadmium phytoremediation by these two plants using inoculating of some bacteria has been reported.

Therefore, both *C. comosum* and *C. amaniense* were selected to use in this study. This research focused on the investigation of the performance of cadmium resistant bacteria on promoting the efficiency of cadmium phytoremediation by *C. comosum* or *C. amaniense* which were planted in cadmium contaminated soil.

Objective of the study

The aim of this study was to investigate the performance of cadmium resistant bacteria on promoting the efficiency of either *C. comosum* or *C. amaniense* on cadmium phytoremediation.

Materials and methods

1. Preparation of cadmium resistant bacteria

Two strains of cadmium-resistant bacteria, namely *Micrococcus* sp. MU1 (Figure 1a) and *Arthrobacter* sp. TM6 (Figure 1b) were cultured in LB broth and incubated at 28°C. Overnight cells of each cadmium resistant bacterium were cultivated in a fresh LB broth in 250-mL Erlenmeyer flask and adjusted the cell density to give an OD_{600} ~ 0.1 measuring by spectrophotometer. All flasks were incubated at 28°C at 150 rpm for 24 h and adjusted the cell density to give an OD_{600} ~ 0.2 using a sterile 0.85% sodium chloride. Each bacterial inoculum was used to inoculate in cadmium contaminated soil in the pot experiments.

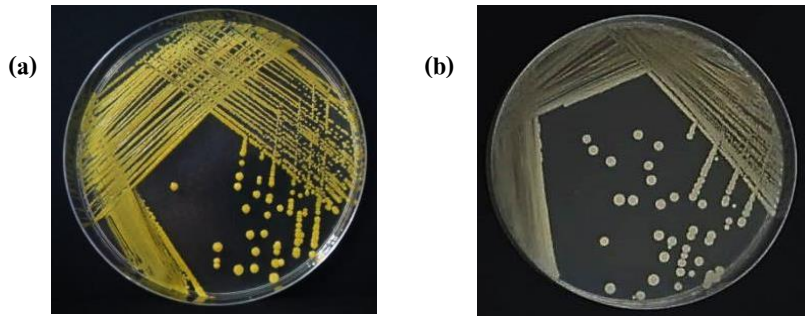

Figure 1 Cadmium resistant bacterial strains; (a) *Micrococcus* sp. MU1 and (b) *Arthrobacter* sp. TM6

2. Preparation of *C. comosum* and *C. amaniense* seedlings

Seedlings of 1-month-olds of *C. comosum* and *C. amaniense* were bought from agriculturalist. After that, seedlings were planted in uncontaminated agricultural soil. The average height and fresh weight of *C. comosum* were 52.8 ± 5.0 cm and 45.3 ± 8.3 g, respectively. The average height and fresh weight of *C. amaniense* were 56.8 ± 7.4 cm and 31.3 ± 7.2 g, respectively. Plants were in the greenhouse for at least 2 weeks before transplanting in cadmium contaminated soil.

3. Experimental study in the pot trial in the greenhouse

To investigate the performance of cadmium resistant bacteria on promoting the cadmium phytoremediation efficiency, each plant species was transplanted in 25-cm-diameter of plastic pots containing 3.0 kg of cadmium contaminated soil. All pots were placed in a greenhouse under natural sunlight and ambient temperature and all plants were watered twice a week in the morning. The pot experiments were designed by completely randomized design and each plant species was divided into 4 treatments with 3 replicates. The details of each treatment present in Table 1.

Table 1 Experimental design of cadmium phytoremediation in the greenhouse study

Treatment	Condition
C. comosum	
TC1	*C. comosum* cultivated in cadmium contaminated soil (the uninoculated control)
TC2	*C. comosum* cultivated in cadmium contaminated soil + 1% (v/w) MU1
TC3	*C. comosum* cultivated in cadmium contaminated soil + 1% (v/w) TM6
TC4	*C. comosum* cultivated in cadmium contaminated soil + 0.5% (v/w) MU1 + 0.5% (v/w) TM6
C. amaniense	
TA1	*C. amaniense* cultivated in cadmium contaminated soil (the uninoculated control)
TA2	*C. amaniense* cultivated in cadmium contaminated soil + 1% (v/w) MU1
TA3	*C. amaniense* cultivated in cadmium contaminated soil + 1% (v/w) TM6
TA4	*C. amaniense* cultivated in cadmium contaminated soil + 0.5% (v/w) MU1 + 0.5% (v/w) TM6

4. Plant and soil analysis

Plant samples in each pot were harvested after transplantation for 3 weeks. Plant growth was measured for stem height and root length. Plants were separated into two parts, including the shoots and the roots. Each plant part was oven-dried at 80˚C before weighting of plant dry biomass. To analyze cadmium concentration in plants, a 0.5 g of dried plant sample was digested with 10 mL of conc. HNO_3 using the microwave digestion. To analyze cadmium concentration in soil, soil sample was oven dried at 103°C and weighted about 0.5 g. 0.5 g of soil was acid digested with 3 mL of conc. HNO_3 and 9 mL of conc. HCl using the microwave digester. To analyze the concentration of bioavailable cadmium, soil was extracted by DTPA solution (Faust and Christians, 2000). In addition, cadmium concentrations in soil and each part of plants and the bioavailable cadmium concentration were determined using Flame Atomic Absorption Spectrophotometry (FAAS). The performance of plant on cadmium uptake and translocation were evaluated using the values of phytoextraction coefficient (PEC) (Kumar *et al.*, 1995), bioaccumulation factor (BAF) (Khaokaew and Landrot, 2015) and translocation factor (TF) (Mattina *et al.*, 2003) as calculated by these following equations.

$$\text{Phytoextraction coefficient (PEC)} = \frac{\text{Cadmium concentration in a whole plant}}{\text{Total cadmium concentration in soil}}$$

$$\text{Bioaccumulation factor (BAF)} = \frac{\text{Cadmium concentration in whole plant tissue}}{\text{Bioavailable cadmium concentration in soil}}$$

$$\text{Translocation factor (TF)} = \frac{\text{Cadmium concentration in shoots } (C_{shoot})}{\text{Cadmium concentration in roots } (C_{root})}$$

5. Statistical analysis

Data was expressed as means with standard deviation (SD). The obtained data from pot experiments were statistically analyzed using One-way Analysis of variance (ANOVA) at 95% confidence level and used to compare the means of different treatments in cadmium phytoremediation experiments followed by DUNCAN multiple range test significant differences at $p < 0.05$.

Results and Discussion

1. Ability of cadmium resistant bacteria on promoting of the growth of *C. comosum* and *C. amaniense*

After plantation in cadmium contaminated soil for 3 weeks, plant samples of each plant species were harvested and determined plant growth. The results of the growth of plants with bacterial inoculation and uninoculated control are shown in Table 2.

Table 2 Growth performance of *C. comosum* and *C. amaniense* after plantation in cadmium contaminated soil for 3 weeks

Treatment	Root length (cm)	Shoot height (cm)	Dry biomass weight (g)
C. comosum			
TC1	25.33 ± 1.04^{a}	26.93 ± 1.40^{ns}	5.72 ± 0.12^{a}
TC2	29.33 ± 1.15^{b}	25.77 ± 0.75^{ns}	9.08 ± 0.14^{c}
TC3	27.17 ± 2.02^{ab}	28.00 ± 2.65^{ns}	7.25 ± 0.24^{b}
TC4	26.67 ± 1.04^{ab}	27.03 ± 1.33^{ns}	6.07 ± 0.43^{a}
C. amaniense			
TA1	22.33 ± 0.91^{bc}	29.17 ± 0.35^{a}	4.93 ± 0.15^{a}
TA2	24.30 ± 1.55^{c}	29.27 ± 0.25^{a}	6.20 ± 0.06^{b}
TA3	17.77 ± 1.25^{a}	31.80 ± 1.00^{b}	6.11 ± 0.06^{b}
TA4	20.90 ± 0.60^{b}	28.33 ± 1.61^{a}	5.69 ± 0.53^{b}

The means and SD ($n = 3$) followed by the different letter within a same column in each plant species were a significant difference ($p < 0.05$) according to Duncan's multiple range test ± SD. The ns were non-significant difference ($p < 0.05$).

The results in Table 2 found that *C. comosum* and *C. amaniense* with *Micrococcus* sp. MU1 inoculation had the higher the root length and dry biomass weight than those of other treatments. The root lengths and dry biomass weights of *C. comosum* were higher than those of *C. amaniense* in all treatments. In addition, both plants with *Micrococcus* sp. MU1 (TC2 and TA2) and *Arthrobacter* sp. TM6 (TC3 and TA3) inoculation showed the highest total dry biomass weight compared to the uninoculated control. Unfortunately, plants with both bacteria inoculation (TC4 and TA4) had the plant growth both root length and dry biomass similar to the uninoculated control. The explanation would involve the low number of each bacterium inoculated in the soil at the ratio of *Micrococcus* sp. MU1 and *Arthrobacter* sp. TM6 by 0.5% and 0.5% (v/w). According to the research of Prapagdee *et al.* (2013), they has been reported that *Micrococcus* sp. MU1 can produce indole-3-acetic acid (IAA) that is able to stimulate root elongation, promote plant

growth, and cadmium uptake by *Helianthus annuus* L. planted in cadmium contaminated soil. Also, the results showed that *C. comosum and C. amaniense* can grow in cadmium contaminated soil. The typical morphology of *C. comosum* and *C. amaniense* were height about 50 to 60 cm approximately, with slender leaves and elongated, smooth and shiny leaves. The potential of *Micrococcus* sp. MU1 and *Arthrobacter* sp. TM6 on promoting the growth of *C. comosum* and *C. amaniense* planted in cadmium contaminated soil compare with the uninoculated control were evaluated. Figure 2 illustrates the external morphology of *C. comosum* and *C. amaniense* after plantation in cadmium contaminated soil for 3 weeks.

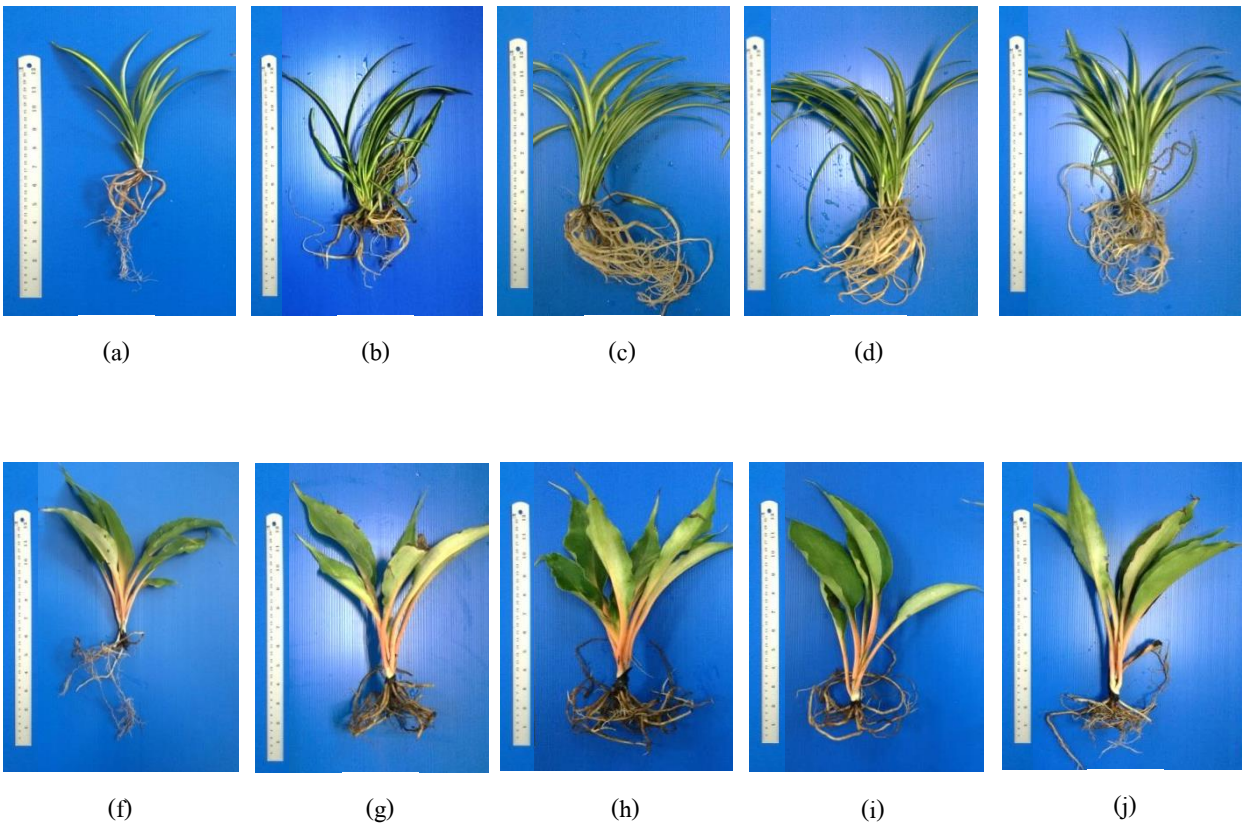

Figure 2 The growth of *C. comosum* and *C. amaniense* after plantation in cadmium contaminated soil for 3 weeks

(a) *C. comosum* before transplanting in cadmium contaminated soil.

(b) TC1: *C. comosum* cultivated in cadmium contaminated soil (the uninoculated control)

(c) TC2: *C. comosum* cultivated in cadmium contaminated soil + 1% (v/w) MU1

(d) TC3: *C. comosum* cultivated in cadmium contaminated soil + 1% (v/w) TM6

(e) TC4: *C. comosum* cultivated in cadmium contaminated soil + 0.5% (v/w) MU1 + 0.5% (v/w) TM6

(f) *C. amaniense* before transplanting in cadmium contaminated soil.

(g) TA1: *C. amaniense* cultivated in cadmium contaminated soil (the uninoculated control)

(h) TA2: *C. amaniense* cultivated in cadmium contaminated soil + 1% (v/w) MU1

(i) TA3: *C. amaniense* cultivated in cadmium contaminated soil + 1% (v/w) TM6

(j) TA4: *C. amaniense* cultivated in cadmium contaminated soil + 0.5% (v/w) MU1 + 0.5% (v/w) TM6

2. Efficiency of *C. comosum* and *C. amaniense* on cadmium accumulation

Cadmium concentration in each part (root, shoot) and whole plant in pot of *C. comosum* and *C. amaniense* after transplantation in cadmium contaminated soil for 3 weeks presented in Table 3.

Table 3 Cadmium contents in each part of plant and a whole plant after plantation in cadmium contaminated soil for 3 weeks

Treatment	Cadmium concentration (mg/kg)		Cadmium concentration of whole plant (mg/kg)
	Root	Shoot	
C. comosum			
TC1	37.50 ± 0.60^{a}	12.31 ± 0.34^{ns}	23.06 ± 0.49^{a}
TC2	40.57 ± 1.26^{b}	13.02 ± 0.46^{ns}	26.73 ± 0.22^{b}
TC3	43.70 ± 1.00^{c}	12.50 ± 0.20^{ns}	29.71 ± 0.66^{c}
TC4	37.80 ± 1.30^{a}	12.45 ± 1.25^{ns}	25.45 ± 1.73^{b}
C. amaniense			
TA1	19.93 ± 3.60^{a}	8.20 ± 2.77^{ns}	11.87 ± 1.29^{a}
TA2	20.13 ± 2.97^{a}	8.87 ± 1.34^{ns}	12.22 ± 0.69^{a}
TA3	29.90 ± 2.91^{b}	10.47 ± 2.08^{ns}	15.90 ± 0.74^{b}
TA4	26.10 ± 4.41^{ab}	6.38 ± 0.22^{a}	15.71 ± 0.79^{b}

The means and SD ($n = 3$) followed by the different letter within a same column in each plant species were a significant difference ($p < 0.05$) according to Duncan's multiple range test ± SD. The ns were non-significant difference ($p < 0.05$).

The results in Table 3 found that cadmium was more accumulated in the roots than the shoot in all treatments of *C. comosum* and *C. amaniense*. Interestingly, *C. comosum* had much more ability to accumulate cadmium in the roots higher than that of *C. amaniense* by 1.9-fold in the uninoculated control treatments. The highest cadmium contents in the roots of both plants were found in the plants with *Arthrobacter* sp. TM6 inoculation (TC3 and TA3), followed by plants of *C. comosum* inoculated with *Micrococcus* sp. MU1 (TC2) and plants of *C. amaniense* inoculated with *Micrococcus* sp. MU1 (TC2 and TA2) and *Arthrobacter* sp. TM6 (TA4). The cadmium contents in the roots of *C. comosum* and *C. amaniense* with mixed culture inoculation (TC4 and TA4) were higher than the uninoculated control treatments of each plant (TC1 and TA1). *C. comosum* with *Micrococcus* sp. MU1 inoculation (TC2) had the highest cadmium content in the shoots compared to other treatments. In addition, plants with *Arthrobacter* sp. TM6 inoculation (TC3 and TA3) had the highest cadmium accumulation in whole plants. Our results indicated that *Micrococcus* sp. MU1 and *Arthrobacter* sp. TM6 can enhance cadmium accumulation in *C. comosum* and *C. amaniense*. According to the study of Khonsue *et al.* (2013),

they found that *Arthrobacter* sp. TM6 can increase cadmium uptake and accumulation in *Ocimum gratissimum* and *Vertiveria nemoralis* planted in contaminated soil. Wang *et al*. (2011) reported the ability of *C. comosum* to remediate cadmium in heavy metal contaminated areas.

3. Cadmium phytoremediation performance of *C. comosum* and *C. amaniense*

The performance of cadmium phytoremediation of plants after plantation in contaminated soil for 3 weeks was expressed in term of phytoextraction coefficient (PEC), bioaccumulation factor (BAF) and translocation factor (TF) as presented in Table 4.

Table 4 Phytoremediation performances of *C. comosum* and *C. amaniense* after plantation in cadmium contaminated soil for 3 weeks

Treatment	Phytoextraction coefficient	Translocation factor	Bioaccumulation factor
C. comosum			
TC1	0.46 ± 0.02^{a}	0.33 ± 0.01^{b}	0.77 ± 0.02^{a}
TC2	0.55 ± 0.04^{b}	0.32 ± 0.02^{b}	0.82 ± 0.01^{a}
TC3	0.64 ± 0.02^{c}	0.29 ± 0.01^{a}	0.91 ± 0.02^{b}
TC4	0.55 ± 0.03^{b}	0.33 ± 0.03^{b}	0.80 ± 0.05^{a}
C. amaniense			
TA1	0.18 ± 0.02^{a}	0.43 ± 0.20^{ns}	0.39 ± 0.04^{a}
TA2	0.21 ± 0.01^{a}	0.45 ± 0.13^{ns}	0.44 ± 0.04^{a}
TA3	0.26 ± 0.01^{b}	0.36 ± 0.10^{ns}	0.55 ± 0.04^{b}
TA4	0.27 ± 0.01^{b}	0.34 ± 0.12^{ns}	0.58 ± 0.05^{b}

The means and SD ($n = 3$) followed by the different letter within a same column in each plant species were a significant difference ($p < 0.05$) according to Duncan's multiple range test ± SD. The ns were non-significant difference ($p < 0.05$).

Table 4 presents the PEC, BAF and TF values of *C. comosum* and *C. amaniense* and found that all values in both plants were quite low. Therefore, our study indicates that *C. comosum* and *C. amaniense* are not cadmium hyperaccumulating plants. In contrast to the study of Wang *et al.* (2012), they reported that *C. comosum* had high bioaccumulation coefficient values and high tolerance to toxicity of cadmium; therefore, it is claimed as a cadmium hyperaccumulating plant. The highest values of PEC and BAF were found in plants inoculated with *Arthrobacter* sp. TM6. BAF values of *C. comosum* inoculated with *Arthrobacter* sp. TM6 (TC3) and *C. amaniense* with mixed culture inoculation (TA4) had higher than that of the uninoculated control. Our study indicated that *Micrococcus* sp. MU1 and *Arthrobacter* sp. TM6 increased PEC and BAF of both plants. In addition, TF values of *C. amaniense* were not significant difference in all treatments. The highest TF value of all plants was found in *C. amaniense* inoculated with

Micrococcus sp. MU1 (TA2). Our finding found that *C. comosum* had higher values of PEC and BAF than *C. amaniense*, indicating that *C. comosum* has more potential to be a cadmium phytoaccumulating plant than *C. amaniense*.

Conclusions

Micrococcus sp. MU1 was able to promote the root lengths and total dry biomass weight of *C. comosum* and *C. amaniense* but it was not promote the shoot heights of both plants. Cadmium was more accumulate in the root than in the shoot of both plants. In particular, *C. comosum* accumulated high amount of cadmium in the roots compared to *C. amaniense*. Both plants with *Arthrobacter* sp. TM6 inoculation had the highest cadmium accumulation in their roots and in the whole plants. *C. comosum* with *Micrococcus* sp. MU1 inoculation had the highest cadmium content in the shoots. Cadmium accumulation in the shoots of *C. amaniense* can be promoted by *Arthrobacter* sp. TM6 inoculation. In addition, inoculation of *Micrococcus* sp. MU1 and *Arthrobacter* sp. TM6 enhanced PEC and BAF of both plants. It could be concluded that a combined use of these cadmium resistant bacteria and *Chlorophytum* spp. enhances cadmium phytoremediation efficiency in contaminated soil.

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