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Pb phytostabilization by fast-growing trees inoculated with Pb-resistant plant growth-promoting endophytic bacterium

Yongpisanphop, J.[1*], Babel, S[2]., Kruatrachue, M[3]., and Pokethitiyook, P[3].

1. Department of Agro-Industrial, Food, and Environmental Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand

2. School of Bio-Chemical Engineering and Technology, Sirindhorn International Institute of Technology, Thammasat University-Rangsit Campus, Pathum Thani 12120, Thailand

3. Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

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ABSTRACT: Inoculation of endophytic bacteria has been accepted as a promising technique to assist phytostabilization of heavy metal-contaminated soils. This study investigated the effects of inoculating a bacterial strain closely related to *Pseudomonas pyschrophila* on the plant growth, and phytostabilization of fast-growing trees *Acacia mangium* and *Eucalyptus camaldulensis*, growing on artificial spiked soil with Pb up to 1500 mg/kg. After 60 days, the results showed that the strain closely related to *P. pyschrophila* slightly increased Pb bioavailability and Pb uptake by *A. mangium*, compared to non-inoculated controls. It slightly reduced Pb bioavailability in soil, but it did not affect the Pb uptake by *E. camaldulensis*, compared to non-inoculated controls. Interestingly, it was able to significantly increase Pb content in shoots by 3.07-fold in *A. mangium* and 2.95-fold in *E. camaldulensis*, compared to non-inoculated controls. Although the inoculation of the strain closely related to *P. pyschrophila* slightly increased the translocation factor (TF) of Pb in both tree species, their TF values were less than 1. This indicates that plants associated with the strain closely related *to P. pyschrophila* are suitable for phytostabilization of *A. mangium*, which may be used for cleaning up Pb contaminated sites. This strain displayed different influences on plant species and was found not suitable for phytostabilization of *E. camaldulensis.*

Keywords: *Acacia mangium*; *Eucalyptus camaldulensis*; Heavy metal; Phytoremediation; *Pseudomonas pyschrophila.*

INTRODUCTION

Heavy metals have specific weight more than 5 g/cm^3 (Ahemad, 2019). The Earth's crust contains lead (Pb) 0.002%, which is categorized to toxic metal class and ranked as the second most toxic heavy metal after arsenic (Zulfiqara et al., 2019). Pb contamination in soils seriously affects terrestrial ecosystems and poses significant risks to human health through its accumulation and transfer in the food chain (Zhang et al., 2011; Ali et al., 2013; Ahemad, 2019). Once Pb enters the soil, it is very difficult to remediate since it has the least bioavailability due to precipitation with soil

[*] Corresponding Author, Email: jiraporn.y@sci.kmutnb.ac.th

components (Saifullah et al., 2009; Ali et al., 2013; Jebara et al., 2015). Thus, the development of remediation strategies for soil contaminated with Pb (particularly by increasing Pb accumulation) is a necessary and challenging task (Jebara et al., 2015). Pb is not easily degraded and persists in soil for a long time. Phytostabilization, using plants to immobilize metals in plant roots and rhizospheres to prevent pollutant migration to groundwater or entry into the food web, is a widespread method to remediate Pb contaminated soils (Pierzynski et al., 2002; Meeinkuirt et al., 2012; Ali et al., 2013; Rajkumar et al., 2013). This strategy is well known as a cheap and environmentally friendly tool for metal contaminated soil remediation.

Recently, there has been increasing interest in cultivating fast-growing trees as energy crops for phytostabilization (Mahar et al., 2016; Pandey et al., 2016; Sylvain et al., 2016). Fast-growing trees have high biomass production and have a large capacity to store heavy metals in their tissues, resulting in more accumulated metals (Sylvain et al., 2016). This can solve environmental sustainability and achieve economic returns as firewood (Pandey et al., 2016). Among fast-growing trees, *Acacia mangium* Willd. and *Eucalyptus camaldulensis* Dehnh. have high heating value with 4900 cal/g, and 4800 cal/g, respectively. Although these trees have the ability to grow in metal contaminated soil, the amount of Pb in plant tissues is limited. Pb in the plants was reported to be around 2 mg/kg (Zulfiqara et al., 2019). Pb causes a number of toxicity symptoms in plants, e.g. stunted growth, decreased dry biomass, chlorosis, necrosis, and blackening of root systems, caused oxidative damages to plants, and induced the structural change in the photosynthetic organelle (Alkhatib et al., 2013; Zulfiqara et al., 2019). These symptoms can impact the potential for Pb phytostabilization, limiting this technology. Supportingly, metal toxicity is the one of major factors limiting the plant growth used in phytoremdiation of metal polluted soils (Fan et al., 2018).

Currently, many researchers have focused on the roles of plant-associated endophytic bacteria with plant growth-promoting trait (PGPTs) in phytoremediation (Shin et al., 2012; He et al., 2013; Babu et al., 2015; Jebara et al., 2015; Ma et al., 2015; Saadani et al., 2016; Navarro-Torre et al., 2016). Endophytic bacteria with PGPTs (e.g., siderophore, indole acetic acid (IAA), exopolysaccharides (EPS), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, hydrogen cyanide (HCN), and ammonia production, N_2-fixation, nitrogenase activity and phosphate solubilization (Ahemad, 2019) can help host plants to adapt to unfavorable soil conditions and enhance the phytoremediation efficiency. This is by promoting plant growth, reducing metal phytotoxicity, altering metal bioavailability in soil, and metal translocation in plants (Ma et al., 2016). However, only a few Pb resistant endophytic bacteria have been reported (Sheng et al., 2008; He et al., 2013). Recently, Yongpisanphop et al. (2019) have isolated the Pb resistant endophytic bacterium strain closely related to *Pseudomonas pyschrophila* (SCRPP). This strain is non-pathogenic and could grow on an Luria-Bartani (LB) agar supplemented with a high Pb concentration of up to 1850 mg/L. It also produced siderophores, solubilized phosphate, and mobilized Pb in soil. The hydroponic study demonstrated that SCRPP could promote Pb phytostabilization of *A. mangium* by increasing the Pb concentration in roots to 6,829±697 mg/kg compared to non-inoculation (6,242±272 mg/kg) (Yongpisanphop & Babel, 2017).

This study investigates the influences of the bacterial SCRPP on plant growth performance, Pb accumulation, and phytostabilization in fast growing trees, *A. mangium* and *E. camaldulensis*, using a pot study.

MATERIALS AND METHODS

The colony of bacteria closely related to *P. pyschrophila* was grown by streaking on an LB agar plate and incubating at 30°C for 48 h. A loop of pure colony was transferred into an LB broth, and incubated on a shaker at 180 rpm, 30±2°C for 48 h as pre-culture. Pre-culture (100 µL) was transferred into 50 mL of LB broth in a flask, and incubated on a rotary shaker at 180 rpm, 30±2°C for 16 h. Bacterial cells were harvested by centrifugation for 20 min at 3,500 rcf at 4°C.

A. mangium and *E. camaldulensis*, were selected based on their demonstrated ability to grow in Pb contaminated soil and their high biomass production (Meeinkuirt et al., 2012). These plants were acclimatized in 25% modified Hoagland's nutrient solution with low phosphate for 7 days. Plants were inoculated by a strain closely related to *P. pyschrophila* using the prune root dip method modified from Bressan and Borges (2004). The roots were washed with sterile distilled water, and they were pruned 10%. All pruned-root plants were washed with deionized water, and weighed for initial fresh weight before inoculation. Then, they were dipped in 300 mL of 25% modified Hoagland's solution with added bacterial inoculum (final density 10^8 CFU/mL) for 3 h. The pruned root plants, dipped in 25% modified Hoagland's solution, were used as controls.

Successful inoculation means that an inoculated SCRPP could colonize inside the roots of new plant hosts. This step needs to be confirmed before performing the pot experiments. The inoculated plants were extracted and endophytic bacteria were isolated by the same method (with isolation from its original plant host) as Yongpisanphop et al. (2019). After incubation, the characteristics of colonies were observed. If circle and milky white colonies were found, this indicated that the SCRPP successfully entered and colonized inside the roots of new hosts. In addition, to ensure that the inoculated SCRPP was not a native species of *A. mangium* and *E. camaldulensis*, non-inoculated plants were also subjected to the same technique as inoculated plants.

Non-contaminated soil was obtained from Suphanburi province. The soil was crushed to remove rocks and debris and passed through a 2x2 mm sieve. It was thoroughly mixed with $Pb(CH_3COO)_2 \cdot 3H_2O$ solution to achieve a final Pb concentration of 1500 mg/kg. The soil without Pb was used as controls (un-contaminated soil). These soils were equilibrated in plastic containers for 15 days. The physico-chemical properties of Pb-spiked soil were: soil texture (hydrometer) clay loam with 38% sand, 34% silt, and 28% clay; pH (1:1, w/v water) 6.8; electrical conductivity (electric conductivity meter) 3.27 dS/m; cation exchange capacity (leaching with 1N NH_4OAc and distillation) 31.6 cmol/kg; organic matter (Walkley and Black method: Walkley & Black, 1947) 6.1%, available P (Bray II method: Bray & Kurtz, 1945) 563 mg/kg; exchangeable K, Ca, and Mg (extracting with 1N NH_4OAc pH 7.0) 1092, 6503, and 545 mg/kg, respectively; and total N (semi micro-Kjeldahl method: Fawcett, 1954) 0.14%.

A piece of filter paper was put at the bottom of a plastic pot (9 inches in diameter, 7 inches in height) to retain the soil and to prevent Pb and endophytic bacteria from leaking out into the environment. Equilibrated soils (2 kg) were added to each pot. Then, all plants were transplanted into a pot (1 plant/pot). There were 4 treatments: *A. mangium* without bacteria, *A. mangium* with bacterial inoculation, *E. camaldulensis* without bacteria, and *E. camaldulensis* with bacterial inoculation. Each treatment was done in triplicate. The plants were grown in a greenhouse for 60 days (July-September 2016) under natural conditions (an average temperature of 35°C and 46%

mean relative humidity). All pots were watered by tap water every 2 days.

After 60 days, the plants were carefully removed from the pots. They were thoroughly washed in running tap water until there were no soil particles adhering to the roots, rinsed with deionized water twice, and weighed as the final fresh weight. To determine the Pb concentration in plant tissues, samples were oven-dried at 60°C for 3 days before determining dry weights. Dried plants were separated into shoots and roots, and they were ground to a powder. Dried plant tissues (0.5 g) were extracted with 10 mL of 69% HNO_3 in a microwave oven (Mars6, U.S.A) (Enamorado-Báez et al., 2013). The operational conditions and the heating program were carried out according to the manufacturer's instructions: a ramp time of 10 min to reach 200°C, then a hold time of 10 min. The digested solutions were filtered using Whatman filter paper No. 42, and the volume adjusted to 100 mL with deionized water. Pb concentrations in the extracts were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES: Optima 8000: PerkinElmer, USA).

Soils were collected at day 0 and day 60 to determine total Pb concentration in soil. The soils were sieved and dried at 60°C for 3 days. Dried soil (0.5 g) was digested with 10 mL of HNO_3 using microwaves (Mars6) according to the method of US-EPA 3051A (US-EPA, 2007). The digested solutions were filtered, and the Pb concentrations in the extracts were determined as described in Section 2.6. Diethylene triamine pentaacetic acid (DTPA)-extractable Pb in soils was also determined according to the method of Lindsay and Norvell (1978). Soil samples were sieved through 0.28 mm nylon sieve and air-dried for 2 days. Dried samples (10 g) were extracted with 20 mL of DTPA solution containing 0.005 M of DTPA, 0.01 M of $CaCl_2{\cdot}2H_2O$, and 0.1 M of triethanolamine (TEA). The pH was adjusted to 7.3 ± 0.05 with 1 N HCl. Samples were horizontal shaken (approximately 150 rpm) for 2 h. The extracted solutions were filtered, and the Pb concentrations in the extracts were determined as described in the section for plant analysis.

In response to Pb exposure, the relative growth (RG), bioconcentration factor (BCF), and translocation factor (TF) were calculated according to the following equations:

$$\mathrm{RG}(\%) = \frac{\text{Final fresh biomass (g)}}{\text{Initial fresh biomass (g)}} \text{x} 100$$

$$\text{Pb uptake (mg / plant)} = \text{Pb concentration in plant (mg / g) x dry biomass (g / plant)}$$

$$\mathrm{BCF} = \frac{\text{Pb concentration in whole plant (mg / kg)}}{\text{Initial Pb concentration in soil (mg / kg)}}$$

$$\mathrm{TF} = \frac{\text{Pb concentration in shoot (mg / kg)}}{\text{Pb concentration in root (mg / kg)}}$$

All data were expressed as the mean and standard deviation (mean ± S.D.) of the three replicates. Independent sample *t*-tests ($p \leq 0.05$), or one-way analysis of variance (ANOVA) followed by the *post hoc* Fisher Least Significant Difference test ($p \leq 0.05$), were used to compare the treatment means. All statistical analyses were carried out using SPSS version 16 for Windows.

RESULTS AND DISCUSSION

Endophytic bacteria, used in plant-assisted phytoremediation are required to have various beneficial traits and excellent colonization ability (He et al., 2013). These traits are crucial parameters for phytoremediation, as some metabolites are produced by a single organism or produced by a plant associated with microorganisms (Mesa et al., 2015). Hence, colonization of the inoculated endophytic bacteria in the root tissues of host plants needs to be observed. The present results showed that the SCRPP was successfully colonized inside the roots of *A. mangium* and *E.*

canaldulensis, as circular and milky white colonies were found. The SCRPP was cultured on an LB agar with 20 mg/L of added Pb as Pb acetate trihydrate [$Pb(CH_3COO)_2 \cdot 3H_2O$]. This implies that the SCRPP, can enter and colonize inside the roots of hosts after inoculation (pictures are not shown here)**.**

Endophytic bacteria promote plant growth in Pb contaminated soil, making them a preferred choice for microbial-assisted phytoremediation studies (Sheng et al., 2008). The influences of the SCRPP on initial and final fresh weight of *A. mangium* and *E. camaldulensis* are shown in Figure 1 and 2, respectively. There were no significant changes ($p > 0.05$) in almost all treated plants, except *A. mangium* (non-inoculation), grown in Pb contaminated soil. These results are in agreement with Ma et al. (2011) who found that *Pseudomonas* sp. A3R3 inoculations did not exhibit a great influence on the fresh and dry weight of *A. serpyllifolium* in Ni soils, as compared with non-inoculated plants. Since each individual plant at the initial step was different in size, the relative growth should be considered.

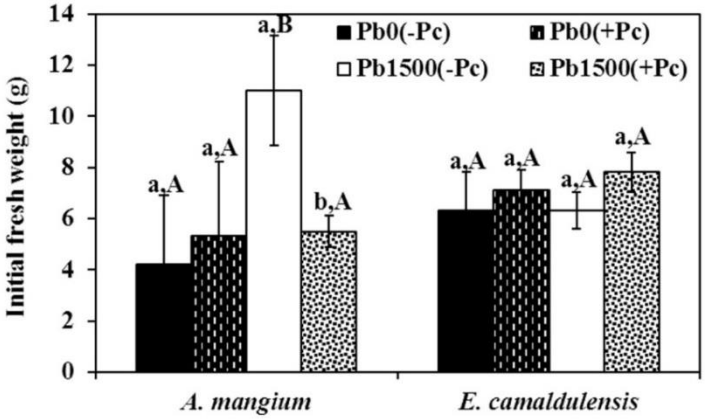

Fig. 1. Influence of the strain closely related to *P. psychrophila* on the initial fresh weight. Error bars represent the standard deviation. The different small letters above the bar graph denote a significant difference ($p \leq 0.05$) between inoculation and non-inoculation. The different capital letters indicate a significant difference of fresh weight between the absence and presence of Pb, as determined by the *t*-test at $p \leq 0.05$. (-Pc) is non-inoculation and (+Pc) is the strain closely related to *P. psychrophila*.

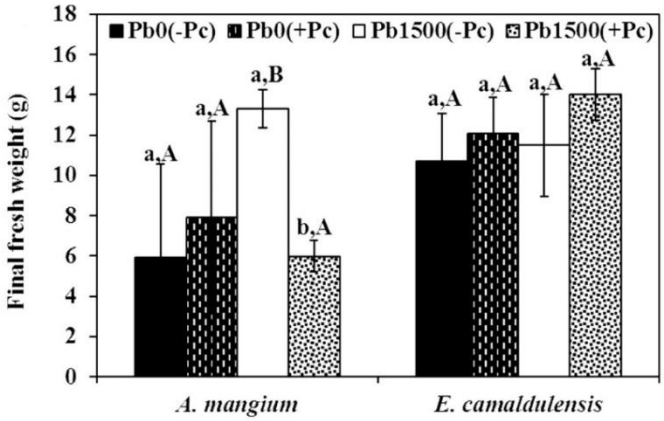

Fig.2. Influence of the strain closely related to *P. psychrophila* on the final fresh weight. Error bars represent the standard deviation. The different small letters above the bar graph denote a significant difference ($p \leq 0.05$) between inoculation and non-inoculation. The different capital letters indicate a significant difference of fresh weight between the absence and presence of Pb, as determined by the *t*-test at $p \leq 0.05$. (-Pc) is non-inoculation and (+Pc) is the strain closely related to *P. psychrophila*.

The influences of the SCRPP on the RG of *A. mangium* and *E. camaldulensis* are shown in Figure 3. In non-contaminated soil, *A. mangium* showed a 0.69% increase in growth with this bacterial endophyte, but *E. camaldulensis* exhibited a 0.58% reduction. In Pb soil, this strain reduced the growth of *A. mangium* by 11.29%, and of *E. camaldulensis* by 1.10 %. These results indicated that there were no significant changes ($p > 0.05$) in all treated plants inoculated by the SCRPP. This can be due to the beneficial effects of bacterial inoculation may be hidden by species-specific interactions between the bacteria or between the bacteria and a plant. The native bacteria and the bacteria introduced by inoculation can compete for space, carbon and nutrients. Such competition could prevent plant growth promotion (Marcos et al., 2016). However, the relative growth was lower in Pb-treated inoculated trees compared to non-inoculated trees. Similarly, the study by Fan and his team found that the inoculation of *Agrobacterium radiobacter* HZ6 and *Mesorhizobium loti* HZ76 with PGPTs lead to decrease of shoots and roots dry biomass of plant (*Robinia pseudoacacia* L.) grown in a mixture of vermiculite and perlite added with 800 mg/kg of Pb as $Pb(NO_3)_2$ (Fan et al.,2018). The growth of sunflower plants inoculated with *Exiguobacterium* sp. RB51 dramatically decreased when grown in Cd-supplemented soil, but shoot and root dry weights were still significantly higher than those of non-inoculated plants. This may be because *Exiguobacterium* sp. RB51 produces IAA and solubilizes phosphate, which can stimulate root elongation and increase soluble phosphate in the soil (Siripan et al., 2018). Certainly, the SCRPP can solubilize phosphate. *Exiguobacterium indicum* SA22 regulated plant growth of *Oryza Sativa* under normal conditions and inhibited all growth parameters when exposed to Cd stress; however, the root-shoot length, fresh, and dry weight were comparatively higher than those of treated non-inoculated plants (Jan et al., 2019).

Recently, there have been many studies that have reported that metal concentrations in plants are closely related to the DTPA-extractable metal concentrations in soils (Ma et al., 2015). In this study, the influence of the SCRPP inoculation on DTPA-extractable Pb concentration in soils was determined after 60 days of growth (Table 1). The inoculation did not significantly influence ($p > 0.05$) the DTPA-extractable Pb in the soil of *A. mangium* and *E. camaldulensis*. Similarly, *Bacillus thuringiensis* HC-2 did not show significant difference ($p > 0.05$) of the DTPA-extractable Pb in the bulk soil of radish (Li et al., 2019).

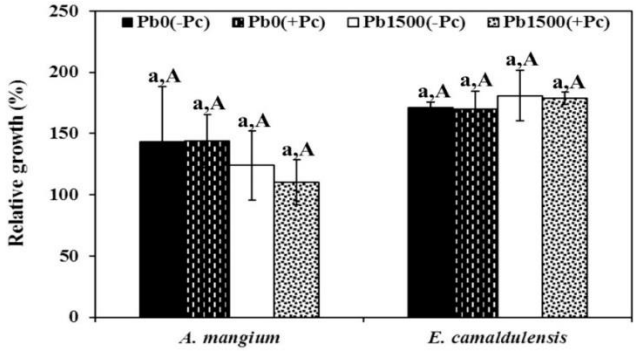

Fig. 3. Influence of the strain closely related to *P. psychrophila* on the relative growth of *A. mangium* and *E. camaldulensis*. Error bars represent the standard deviation. The different small letters above the bar graph denote a significant difference ($p \leq 0.05$) between inoculation and non-inoculation. The different capital letters indicate a significant difference of relative growth between the absence and presence of Pb, as determined by the *t*-test at $p \leq 0.05$. (-Pc) is non-inoculation and (+Pc) is the strain closely related to *P. psychrophila* inoculation.

Table 1. Effects of the strain closely related to *P. psychrophila* inoculation on DTPA-extractable Pb concentration in soil, Pb uptake by plants, bioconcentration factor, and translocation factor of Pb in plants after harvesting

Treatments	DTPA-extractable Pb (mg/kg)	Pb uptake (mg/plant)	BCF	TF
A. mangium (-Pc)	436 ± 65.7[a]	2.11 ± 0.25[a]	0.46 ± 0.18[a]	0.01 ± 0.00[a]
A. mangium (+Pc)	458 ± 51.0[a]	3.20 ± 1.27[a]	0.59 ± 0.04[a]	0.02 ± 0.00[a]
E. camaldulensis (-Pc)	452 ± 15.3[a]	2.00 ± 0.77[a]	0.24 ± 0.06[a]	0.01 ± 0.00[a]
E. camaldulensis (+Pc)	425 ± 20.3[a]	2.02 ± 0.09[a]	0.21 ± 0.04[a]	0.04 ± 0.02[a]

Each value is the mean of triplicate samples ± standard deviation. The mean of each column indexed by the same letter is not significantly different (between inoculation and non-inoculation), as determined by the *t*-test at $p \leq 0.05$. (-Pc) is non-inoculation and (+Pc) is the strain closely related to *P. psychrophila* inoculation.

However, the SCRPP could slightly increase the DTPA-extractable Pb in the soil of *A. mangium* by 5%, but slightly decrease that in the soil of *E. camaldulensis* by 6%. As expected, the inoculation of plants with the SCRPP could promote and reduce heavy metal bioavailability depending on the combination of plant, bacterium, and metal used (Sessitsch et al., 2013). Similarly, plant growth-promoting bacteria (PGPB) such as *Bacillus thuringiensis* HC-2 significantly reduced (approximately 26%) the DTPA-extractable Pb contents in the rhizospheric soil of radish. This can be due to significant increase in the NH_4^+-N content and the NH_4^+/NO_3^- ratio in the rhizosphere soils (Li et al., 2019). The increase of DTPA-extractable Pb concentration after bacterial inoculation is attributed to the solubilization of unavailable forms of heavy metal-bearing minerals. This is due to complexation reactions as a consequence of metabolites (e.g., organic acids, siderophores) released by PGPB (Ma et al., 2015). Henning et al. (2016) also explained that the host physiological response to endophyte inoculation may vary with bacterial strain, plant host, and plant ontogeny.

The inoculation of the SCRPP showed no significant difference ($p > 0.05$) in Pb uptake by plants (Table 1). This may be due to the short exposure time. Normally, Pb uptake by fast-growing trees in field experiments is higher than those in pot experiments. Meeinkuirt et al. (2012) found that *A. mangium* and *E. camaldulensis* could uptake Pb by 1.51 and 36.6 μg/plant in pot experiment, respectively. In a field experiment for 6 months, Pb uptake by *A. mangium* and *E. camaldulensis* was 48.63 and 94.0 μg/plant, respectively, and for 12 months was 797.3 μg/plant by *A. mangium* and 598.7 μg/plant by *E. camaldulensis*. If the duration of exposure in this study was expanded, the results may show higher uptake. Pb uptake was slightly increased in *A. mangium* (52%) and *E. camaldulensis* (1%). The increase of Pb uptake in *A. mangium* could be because the SCRPP increases water-soluble Pb concentrations in soils via siderophore production and phosphate solubilization, leading to improved efficiency of phytoremediation (Ma et al., 2011).

The influences of the SCRPP inoculation in Pb accumulation in *A. mangium* and *E. camaldulensis* are shown in Figure 4 and 5. Regardless of bacterial inoculation, *A. mangium* accumulated more Pb than *E. camaldulensis* in both the root and shoot tissues. In both plant species, the Pb concentrations in the roots (341-988 mg/kg) were much higher than those in the shoots (3.83-16.6 mg/kg). A similar finding was reported by Fan et al. (2018) who found that Pb concentrations in the roots were greater than those in the shoots of *R. pseudoacacia*.

Inoculation of plants with the SCRPP significantly increased Pb accumulations in shoots ($p \leq 0.05$) by 3.07-fold and 2.95-

fold in *A. mangium* and *E. camaldulensis*, respectively (Figure 4). Similarly, *M. loti* HZ76 significantly increased Pb contents in the shoots of *R. pseudoacacia* (Fan et al., 2018). This may be due to bacterial siderophores, as they can translocate Fe from roots to shoots (Luo et al., 2011). Similarly, *Serratia marcescens* (LKR01) isolated from roots of *Solanum nigrum* L. could produce siderophores like these endophytic bacteria. This also improved the translocation from roots to shoots of *S. nigrum*, leading to increased TF value and increasing phytoremediation efficiency (Luo et al., 2011). The increase of Pb accumulation in shoots could be due to the SCRPP's ability to transfer Pb from roots to shoots (Ma et al., 2016).

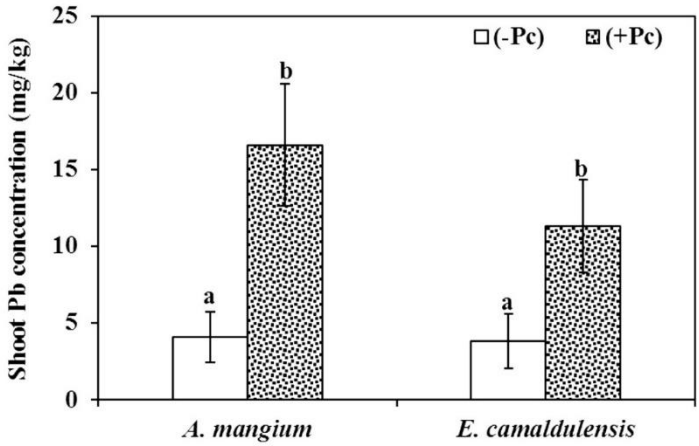

Fig. 4. Effects of the strain closely related to *P. psychrophila* inoculation on Pb accumulation in shoots of plants grown on Pb contaminated soil. Each value is the mean of triplicates. Error bars represent the standard deviation. The different small letters above the bar graph denote a significant difference between inoculation and non-inoculation, as determined by the *t*-test at $p \leq 0.05$. (-Pc) is non-inoculation and (+Pc) is the strain closely related to *P. psychrophila* inoculation.

In this study, there were no significant changes ($p > 0.05$) in Pb accumulation in roots caused by inoculation in both plant species (Figure 5). These results are in accordance with previous research. Sheng et al. (2008) found that there was no significant difference in Pb concentration in roots of *Brassica napus* L. between endophytic bacterial inoculation and non-inoculation, while a significant increase was found for Pb concentrations in shoots of inoculated plants. Also, *M. loti* HZ76 showed no significant difference of Pb contents in the roots of *R. pseudoacacia* inoculated compared to non-inoculated controls (Fan et al., 2018). This SCRPP can increase Pb concentration in roots of *A. mangium*. Similarly, inoculation of *A. radiobacter* HZ6 can promote Pb concentration in roots of *R. pseudoacacia*. The inoculation with PGPB may have relieved metal stress of the plants compared with non-inoculated controls (Fan et al., 2018). In contrast, this bacteria endophyte decreases Pb accumulation in roots of *E. camaldulensis*. This is consistent with previous studies. Endophytic bacteria *Methylobacterium oryzae* and *Burkholderia* sp. reduced metal contents in roots of *Lycopersicon esculentum* due to bacterial immobilization of metals in the rhizosphere (Madhaiyan et al., 2007). Li et al. (2012) reported that metal-resistant endophytes can decrease a plant's metal uptake and accumulation. These endophytic bacteria could promote or inhibit Pb accumulation. The effects of microbial inoculation on the metal extraction capacity of plants depend on the plant species, metal concentration, and microbial strains (Sessitsch et al., 2013).

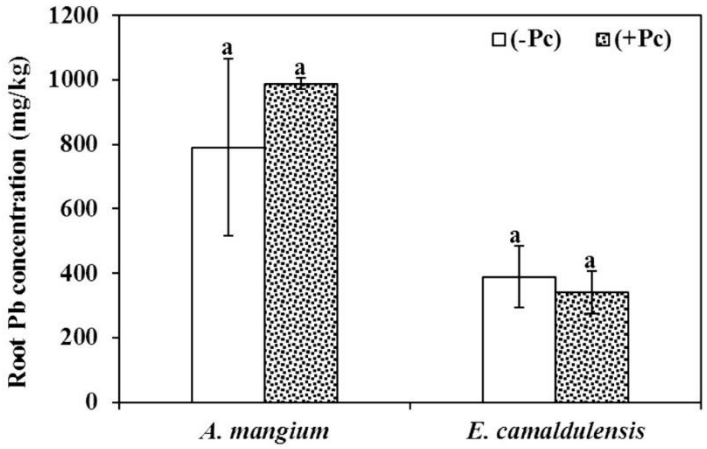

Fig. 5. Effects of the strain closely related to *P. psychrophila* inoculation on Pb accumulation in roots of plants grown on Pb contaminated soil. Each value is the mean of triplicates. Error bars represent the standard deviation. The different small letters above the bar graph denote a significant difference between inoculation and non-inoculation, as determined by the *t*-test at $p \leq 0.05$. (-Pc) is non-inoculation and (+Pc) is the strain closely related to *P. psychrophila* inoculation.

The overall results showed much higher Pb accumulation and uptake of *A. mangium* than *E. camaldulensis* after the bacterial inoculation. Jiang et al. (2008) found that among plants (Indian mustard, maize, and tomato) inoculated with metal mobilizing, PGPB varied in their ability to accumulate heavy metals. Tomato accumulated a higher concentration of heavy metals than other plant species from the soil.

The BCF and TF values of Pb in plants are presented in Table 1. There were no significant changes ($p > 0.05$) in BCF and TF values caused by bacterial inoculation in both species of plants. In *A. mangium*, the SCRPP slightly increased the BCF of Pb by 28%, but the BCF was slightly reduced in *E. camaldulensis* by 12% compared with non-inoculated plants. The inoculation slightly increased the TF values of *A. mangium* and *E. camaldulensis* by 2-fold and 4-fold, respectively. Similarly, Fan et al. (2018) showed % TF of *Robinia pseudoacacia* inoculated with *Agrobacterium radiobacter* HZ6 increased approximately 2-fold compared with non-incoculated control. Ma et al. (2015) found that *Pseudomonas* sp. A3R3 slightly increased the TF values of Ni in *B. juncea* and *Ricinus communis* L., compared to non-inoculated plants. Sun et al. (2010) found that endophytic bacteria enhanced the Cu transfer from roots to the above-ground tissue of *B. napus* growing in Cu-contaminated substrate. The Cu concentration in the aerial part of *B. napus* increased (125%), compared to the non-inoculated control (63%). This indicates that the SCRPP plays an important role in transferring Pb from roots, leading to an increase in Pb accumulation in the shoots of these plants. Similarly, PGPB such as *Pantoea stewartii* ASI11, *Enterobacter* sp. HU38, and *Microbacterium arborescens* HU33 increase Pb accumulation in the shoots of *Leptochloa fusca* (L.) Kunth, compared to non-inoculated plant (Ahsan et al., 2017). The SCRPP could help transfer Pb from roots to shoots. However, the amounts of Pb transported to shoots were small compared to Pb hyperaccumulators. The TF values were less than 1. These accumulators prevent the impact of Pb in the food web. Therefore, Pbtolerant plant growth-promoting endophytic bacteria the strain closely related to *P. psychrophila* can be used for Pb phytostabilization by *A. mangium*.

CONCLUSION

The results conclusively suggest that inoculation with the SCRPP could transfer

Pb from roots to shoots of *A. mangium* and *E. camaldulensis*. However, inoculation of the SCRPP seemed to be effective in promoting the phytostabilization potential of only *A. mangium* based on plant growth, Pb accumulation, Pb uptake, and DTPA-extractable Pb, but it was not useful for *E. camaldulensis*. To the best of our knowledge, this is the first article studying the potential of Pb-resistant SCRPP in Pb phytostabilization by high energy fast-growing tree species. Although our results did not show a significant difference, however it opens a perspective for creating a system of utilizing fast-growing trees with high energy associated with local Pb-resistant endophytic bacterial strains to remediate Pb contaminated soil. It is important to note that the field experiments with a long-time association need to be investigated for further work to confirm the efficiency of Pb phytostabilization in soil as a biological tool for better ecology and economy.

GRANT SUPPORT DETAILS

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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