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Reduction of cadmium uptake in rice endophytically colonized with the cadmium-tolerant bacterium *Cupriavidus taiwanensis* KKU2500-3

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Abstract

The effects of the cadmium (Cd)-tolerant bacterium *Cupriavidus taiwanensis* KKU2500-3 on the growth, yield and Cd concentration in rice grains were investigated in the rice variety Phitsanulok 2 (PL2), which was cultivated in a hydroponic greenhouse. The numbers of Cd-tolerant bacteria isolated from the roots and shoots of plants under the RB [rice with bacteria] and RBC [rice with bacteria and Cd] treatments ranged from 2.60 to 9.03 and 3.99 to 9.60 log CFU·g⁻¹ of PL2, respectively. This KKU2500-3 strain was successfully colonized in rice, indicating that it was not only non-toxic to the plants but also became distributed and reproduced throughout the plants. SEM analysis revealed attachment

of the bacterium to the root surface, whereas the internally colonized bacteria were located in the vascular tissue, cell wall and intercellular space. Although the Cd contents found in PL2 were very high (189.10 and 79.49 mg·kg⁻¹ in the RC [rice with Cd] and RBC roots, respectively), the Cd accumulated inside the rice seeds at densities of only 3.10 and 1.31 mg·kg⁻¹, respectively; thus, the bacteria reduced the Cd content to 57.74% of the control content. Therefore, the colonizing bacteria likely acted as an inhibitor of Cd translocation in PL2.

Keywords: *Cupriavidus taiwanensis* KKU2500-3, cadmium, rice, colonization, hydroponic system

Introduction

Rice has long been an important crop in Thailand and several other countries. At present, rice production is confronted with risks stemming from several factors, especially farmland contamination by pollution. A report from the International Water Management Institute (IWMI) and the Department of Agriculture indicated that harmful Cd contamination levels were present in the soil and rice obtained from paddy fields in the Mae Tao River Basin of the Mae Sot district (an area near zinc mining and a zinc smelting plant). The report suggested that the use of irrigated water for cropping was the source of Cd uptake in the crop plants and concluded that the Cd levels in rice consumed at home would likely lead to Cd toxicity in the exposed population (Simmons et al. 2005).

Cd is a heavy metal that is considered a major pollutant of the water distributed in aquatic environments (Lockwood 1976). Serious Cd problems were noted downstream of a zinc mine and were attributable to smelting processes and mining waste, which released Cd into the groundwater and caused serious pollution of the downstream soil, especially the

paddy fields, water, crop plants and aquatic animals and humans living in the contaminated areas and consuming the toxic rice and food (Yanagisawa et al. 1984). Cd is also considered one of the top toxic metals causing renal injury, lung weakness, bone lesions, cancer, anemia, hypertension and Itai–Itai disease (Ishihara et al. 2001; Qi and Aldrich 2008; Rocha et al. 2009; Farooq et al. 2010).

Cd pollution is a serious problem worldwide. Large regions of contaminated land in areas including China (Gu and Zhou 2002; Yu, et al. 2006) and Japan (Yanagisawa et al. 1984; Ishihara et al. 2001; Nogawa et al. 2004) are being used to produce foodstuffs, which increases the risk of poison contamination in the human food chain. Cd concentrations found in rice samples produced in hazardous areas in Thailand ranged from 0.007 to 0.579 mg·kg⁻¹ (Zwicker et al. 2010). Sriprachote et al. (2012a) determined that the Cd contents in rice grains consumed at home in 23 households in the village of Pha Te, Mae Sot District, were in the range of 0.04 to 1.75 mg·kg⁻¹. Pluemphuak et al. (2014) also reported that the amount of Cd in rice growing around the Mae Tao River floodplains ranged from 7.15 to 14.24 mg·kg⁻¹, indicating that most of the rice samples had levels higher than the CODEX standard (maximum of 0.4 ppm mg/kg), according to the Codex Alimentarius Commission (2006). This situation is of serious concern, leading to a consumer demand for safe and clean foods and attracting the interest in alternative techniques to produce Cd-free agricultural products. Therefore, determining a suitable rice cultivar that takes up less Cd, for the cultivation of rice plants with lower Cd concentrations in their grains, is crucial.

Bioremediation using microorganisms to clear (break down, convert, neutralize and/or remove) contaminants from contaminated sites has advantages over other methods since the contaminated soil is handled onsite without a requirement for excavation, pumping or transfer to other treatments. Although this technique represents a good cleaning method for some types of pollutants, it does not work in all cases. For instance, bioremediation may not

be achievable for sites contaminated with high levels of contaminants, especially Cd, lead (Pb) or sodium chloride, which are toxic to most microbes.

Under these circumstances, phytoremediation (the application of plants to extract, sequester, and/or detoxify contaminants) is more suitable. Phytoremediation exploits the ability of plants to accumulate the toxic substances in their harvestable parts, which can be disposed of without affecting the biological activity, structure and fertility of the soil (Rajkumar et al. 2012). Based on plant-microbe interactions, efforts are being made to combine the capabilities of the two techniques; indeed, many studies are trying to harness the ability of bacteria to act as “bioremediators” to treat contaminated areas in combination with plants that act as “hyperaccumulators” to reduce toxic metal contamination in air, soil and water. The success of this technique is based on a scenario in which plants can fight against the poison of heavy metals and fully grow and accumulate a large amount of biomass and the target metals (Rajkumar et al., 2012). Bacteria that can perform under these conditions are termed “plant growth-promoting bacteria”. This process will directly change the accumulation of heavy metals in plant tissue and indirectly enhance the biomass construction of the plant (Belimov et al. 2005; Rajkumar et al. 2012).

The prominent working mechanism of these bacteria in hyperaccumulating plants is to increase the bioavailability or solubility of heavy metals and thus allow the plants to absorb these target contaminants more easily. Possible mechanisms include the creation of various organic compounds, including several types of siderophores (Tripathi et al. 2005; Dimkpa et al. 2009), organic acids (Wani et al. 2007; Arwidsson et al. 2010), biosurfactants (Juwarkar et al. 2007; Sheng et al. 2008; Venkatesh and Vedaraman 2012) or oxidation and reduction reactions (Shi et al. 2011; Yang et al. 2012). These mechanisms increase the mobilization and accumulation of toxic metal in the plants. These bacteria can also use other mechanisms to alter the solubility of metals and reduce their movement into the plant. Examples of these

mechanisms include the production of cell wall lipopolysaccharides or extracellular polymeric substances, which immobilize and reduce Cd and chromium (Cr) absorption (Joshi and Juwarkar 2009). Polymeric substances and glycoproteins such as glomalin can restrain and detoxify copper (Cu), Pb and Cd (Gonzalez-Chavez et al. 2004).

Cupriavidus taiwanensis (formerly *Ralstonia taiwanensis*) was originally isolated from root nodules of *Mimosa pudica* and *Mimosa diplotricha* (Chen et al. 2001; 2003; 2005). These bacteria have infected and nodulated a wide range of *Mimosa* species via root hairs and were shown to be effective N₂-fixing symbionts of *Mimosa* (Chen et al. 2003; 2005; Elliott et al. 2007). Several species of the *Cupriavidus* genus are metal-tolerant microorganisms (Chen et al. 2008; Klonowska et al. 2012; Siripornadulsil and Siripornadulsil 2013; Platero et al. 2016), and some of these bacteria have been proposed to be used as bioremediators to reduce the toxicity of heavy metals in plants. *C. taiwanensis* TJ208 symbiosis with *M. pudica* has been developed to remove heavy metals based on the metal adsorption capacity of the plant-microbe combination (Chen et al. 2008). *C. metallidurans* and *C. necator* enhance the growth and phytoremediation properties and reduce Cd accumulation in sunflower plants (by up to 64%) growing in contaminated areas (Rozycki and Nies 2008; Marques et al. 2013). *C. taiwanensis* EJ02 responds to Cd stress by producing polyhydroxybutyrate (PHB) and tolerates Cd at high Cd concentrations of up to 5 mM (Chen et al. 2014). *C. metallidurans* exports toxic metal ions from its cell via heavy metal exporters that are part of the transenvelope protein complex (von Rozycki and Nies 2008).

The Cd-tolerant bacterium *C. taiwanensis* KKU2500-3 was isolated from the rhizosphere of rice grown in the high Cd-contaminated area of the Mae Sot District and exhibits several potential properties. This bacterium was able to tolerate to 2,500 µM Cd, bind up to 6.38 log [Cd (atom)] cell⁻¹ and transform toxic CdCl₂ into non-toxic CdS.

Interestingly, it exhibited an interaction with rice via reducing the translocation of Cd and enhanced some rice growth parameters when grown under high Cd concentration (Siripornadulsil and Siripornadulsil 2013). Thus, this bacterial strain exhibits the capability to detoxify toxic heavy metals and promote rice growth under Cd stress, although the mechanism is unknown. Previous studies have not investigated rice plants grown at the mature stage and their production. Therefore, this study focused on the efficacy of *C. taiwanensis* KKU2500-3 for the reduction of Cd in rice grains. The completion of this work may not only create a new bioremediation technology with a minimal impact on the environment while reducing and/or removing Cd from rice grains, paddy fields, and soil and water resources but may also create a product with acceptable quality from a contaminated area. These developments will contribute to the successful improvement of rice cultivation in many hazardous areas worldwide.

Materials and Methods

Materials

Phitsanulok 2 (PL2) rice seeds (a high-yielding and non-glutinous rice cultivar that is not sensitive to the photoperiod and is generally grown in the North and other irrigated areas of Thailand) were kindly provided by the Khon Kaen Rice Research Center, Khon Kaen, Thailand. *C. taiwanensis* KKU2500-3 was isolated from a Cd-polluted paddy field in Mae Sot, Tak Province (Siripornadulsil and Siripornadulsil 2013)

Colonization of *C. taiwanensis* KKU2500-3 in PL2 rice

The PL2 rice seeds were serially surface sterilized with 90% ethanol for 3 min, incubated in 0.2% mercuric chloride for 30 min and then washed with sterile distilled water 3 times. Three days after germination (DAG) in sterile distilled water, the rice seedlings

were separated into two sets; the first set, which was a control set, was not colonized with *C. taiwanensis* KKU2500-3, and the second set was inoculated with 5×10^6 cells/mL of the bacterium for 2 days. The trials were performed under controllable conditions in a growth chamber (70% relative humidity, a 16:8 h light–dark cycle, and $1,000 \mu\text{mol m}^{-2}\text{s}^{-1}$ of light intensity) for 15 days after transplanting (DAT) at 28°C prior to planting in a hydroponic greenhouse (Punjee 2016).

Transplantation of rice into the hydroponic system and the treatments

The two sets of 15 DAT seedlings were separated into 2 groups as follows: R (rice without bacteria) and RB (rice colonized with bacteria). All of the rice seedlings were grown separately in (i) a growth chamber to determine the colonization of bacteria under sterile conditions with semisolid Hoagland's solution (Hoagland and Arnon, 1950) via scanning electron microscopy (SEM) and (ii) a hydroponic system containing Hoagland's solution to track the colonization traits of the bacteria in rice plants using plate counting and 16S rDNA sequence analysis. At 29 DAT, CdCl_2 was added to one half of both treatments, and the Cd concentration was adjusted to 5 μM to establish the RC (rice supplemented with Cd) and RBC (rice colonized with bacteria and supplemented with Cd) treatments.

Bacterial colonization determination

The inoculated rice plants (RB and RBC) grown under sterile conditions were uprooted at 30 DAT, while those grown under hydroponic conditions were uprooted once per month. The rice plants were separated into 3 parts (roots, shoot base and leaves or shoot tips) and used to assess colonization on the exterior and the interior of the roots, shoot base and shoot tips. To evaluate bacterial colonization on the root exterior, the roots were excised, washed with sterile distilled H_2O to remove loosely associated bacteria and then

shaken vigorously with a vortex in sterile normal saline for 30 sec to detach the rhizospheric bacteria. The suspension was diluted with sterile normal saline (10-fold dilution series) and spread onto nutrient agar containing 500 μM CdCl_2 . The Cd-tolerant bacteria that colonized the plants were expected to be *C. taiwanensis* KKU2500-3; these bacteria were counted as CFU/g per plant and identified via 16S rDNA sequence analysis.

Bacterial colonization in the interior of the plants was assessed using the same vortexed plant materials described above. The excess normal saline was then mechanically wiped off the plant materials with sterile soft paper. The clean and dried plant materials were ground with a mortar and pestle in normal saline, and 10-fold serial dilutions were prepared in normal saline. Each dilution was spread onto nutrient agar containing 500 μM CdCl_2 . The Cd-tolerant bacteria that colonized the plants were expected to be *C. taiwanensis* KKU2500-3; the bacteria were counted in CFU/g per plant and identified via 16S rDNA sequence analysis.

Bacterial identification by 16S rDNA sequencing

A 16S rDNA-based PCR technique was performed using optimized conditions (pre-denaturation, 94°C/3 min; denaturation, 94°C/30 s; annealing, 66°C/30 s; extension, 72°C/1.30 min; and post-extension, 72°C/3 min). We designed specific primers to detect the colonized bacteria isolated from the plant samples that were expected to be *C. taiwanensis* KKU2500-3 (CD3-16S rDNAF: 5'GGGACCGCAAGGCCTCGCGC-3' AND CD3-16S rDNAR: 5'-CTCCCCCTCGCGGGTTGGCAA-3'). The probable size of the resulting band was 1,071 base pairs. The DNA fragments were visualized by gel electrophoretic analysis, and the PCR product sequence was aligned with the known 16S rDNA sequence of *C. taiwanensis* KKU2500-3. A BLAST search was performed using the NCBI database.

SEM analysis

Rice plants from the 4 treatments (R, RB, RC and RBC) were collected at 30 DAT and washed gently with sterile water two times. Then, the samples were immersed in glutaraldehyde and paraformaldehyde in sodium cacodylate buffer (pH 7.2) at 28°C for 2 h and washed three times with phosphate-buffered saline (PBS, pH 7.2). The plant tissues were subsequently cut to separate the shoots and roots and soaked in osmium tetroxide in PBS at 4°C for 2 h, followed by dehydration in an ethanol series (50-100%) and desiccation via critical point drying and sputter-coated with gold-palladium (modified from Mattos et al. 2008). Observations and micrographs were performed using SEM (LEO model 1450VP) with an accelerating voltage of 12 kV.

Rice growth and yield determinations

Rice samples from all of the treatments were randomly collected at 30-135 DAT to determine growth based on the biomass analysis, shoot and root lengths and pigment contents. After transplanting every 15 days, the rice leaves were cut into small pieces. The pigments were extracted with 80% acetone, and the absorbance was measured at various wavelengths using a spectrophotometer. The amounts of pigments were computed (Lichtenthaler and Buschmann 2001). The grain and components of the rice yield were evaluated at the end of the experiment (135 DAT).

Cd accumulation and distribution in hydroponic rice

After transplanting for 30, 45, 60, 75, 90, 105, 120 and 135 days, the dried rice samples were cut to separate the root, shoot and grain. The Cd accumulation and distribution in the plant parts were evaluated using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) at the Central Laboratory of Khon Kaen University. Cd

accumulation in the root, shoot, or grain from the non-inoculated and inoculated rice was compared to assess the transport and accumulation of Cd in the rice.

Translocation factor (TF)

Cd translocation from the root to the shoot of the rice plants was determined using the TF.

$$TF = [Cd_{shoot}] / [Cd_{root}]$$

where $[Cd_{shoot}]$ and $[Cd_{root}]$ were the Cd concentrations in the shoots and roots ($mg \cdot kg^{-1}$), respectively (Zhang et al. 2002; Fayiga and Ma 2006). The TF level indicated the movement of Cd into the rice plant and the capability of the plant to act as an accumulator ($TF > 1$) or excluder ($TF < 1$) of the metal (Lorestani et al. 2011; Rezvani and Zaefarian 2011).

Statistical analysis

Data from each experiment were examined using ANOVA, and the mean $\pm$ SD was compared by Duncan's multiple range test (DMRT). Significant levels were reported as $p \leq 0.05$.

Results

Bacterial colonization and/or distribution in the PL2 rice

In PL2 rice, the distribution of bacteria inside and outside of the plant differed. The RB rice showed bacterial spread on the root over the shoot. The most bacteria were found on the root surface during the first 30 DAT. The number of bacteria found in the root was less than the number found outside or on the root surface but was greater than the number found in the shoot base and tip (Fig. 1RB). Compared to the RB rice, the RBC rice had a high number of colonizing bacteria throughout all parts of the crop (Fig. 1RBC). The

bacteria on the root surface tended to decrease as the crop aged, whereas the number of bacteria within the root remained relatively constant from 60-120 DAT. This finding was in contrast to the trend of the distribution of the bacteria into the interior of the shoot base, which was increased at 60 DAT and increased even more in the tips of the crop (Fig. 1).

All of the isolates contained the 16S rDNA sequence of *C. taiwanensis* KKU2500-3. The DNA sequencing results confirmed that the Cd-tolerant bacteria found in the hydroponic PL2 rice was from the KKU2500-3 strain, based on a sequence identity of at least 99%. The colonizing bacteria were visualized by SEM analysis. No bacteria were observed in any tissues of the uninoculated R and RC plants (Figs. 2 and 3), while localization of the bacteria in the inoculated plant tissue (RB and RBC) was evident. First, bacteria were found on the root surface and in the root interior of RB and RBC (Fig. 2). After the bacteria entered the root cells, some of them may have traveled to aerial parts of the plant and localized to the shoot bases and shoot tips (Fig. 3). Their distribution was concentrated in the xylem (x), and the bacteria adhered to the cell walls of the vascular tissue of shoot bases and shoot tips (Figs. 3RB, RBC).

Growth and pigment content of PL2

Although the lengths of the RB rice only slightly differed from the lengths of the R rice (Fig. 4A), the dry matter was significantly increased, especially from 90 DAT until harvest (Fig. 4C). The root length and root dry weight were also increased (Fig. 4B and 4D). Moreover, the RC rice was likely to thrive until yield, but the growth of the shoot was less than the growth of the root due to the reduction in the shoot length and its dry weight compared with the shoot length and dry weight of the control (Fig. 4C). The RBC rice grew well, especially the root, with a length equivalent to that of R and a dry weight close to that of R (Fig. 4D). Although the addition of 5 μM CdCl_2 decreased the total length and the

shoot dry weight, these effects were not expected to be harmful to the yield. Therefore, Cd resulted in inhibition of the shoot rather than the root growth of PL2.

In comparison, the amount of pigments in the leaves of PL2 decreased in the presence of lower concentrations of Cd (Fig. 5). All of the pigments were increased in the RB rice, especially chlorophyll a, with growth times similar to those of the control (Fig. 5). Although the pigment in RBC was less than the amount of pigment in the control, the pigment tended to increase with growth, which was in contrast to the results found in the RC rice (Fig. 5).

PL2 yield

PL2 products planted in the presence of the bacterium had a higher yield (Table 1RB) than the control rice grown without the bacterium (Table 1R). The RB rice produced approximately three tillers per plant, whereas the R rice produced two shoots per plant, which was equivalent to the RBC rice. Furthermore, the bacteria caused the panicle to grow to a greater length, and more rice grains per panicle and a higher filled grain and grain weight were observed in the RB rice than in the control. For the RC products, although the rice was grown until harvest, the effects of Cd resulted in a reduction in growth and the pigment content of the PL2 rice. These effects had consequences for the formation of the tiller, which dropped to approximately one tiller per plant, and resulted in reduced yield components, particularly the production of more unfilled grain. The most interesting aspect of this study was the ability of *C. taiwanensis* KKU2500-3 to promote the development of the tiller, panicle length and number of grains per panicle similar to the R rice (Table 1). Moreover, the number of grains per panicle increased approximately two-fold and the filled grain percentage increased four-fold compared with that of the RC rice (Table 1). Interestingly, the weight of the grain was similar and tended to be higher than that of the control (R).

Cd accumulation in PL2 rice

The amount of Cd in the roots of rice subjected to both the RC and RBC treatments was higher than the amount of Cd in the shoots (Fig. 6). The total Cd accumulation in the RC rice was significantly higher than the accumulation in the RBC rice, especially at 90 DAT, and then dropped at 120 DAT. Although the root absorbed Cd in high doses on the first day (30 DAT) in the RBC rice, the amount of Cd decreased over the first month until there was no difference from the total Cd concentration in the shoot. Then, the accumulation of Cd increased between 90 and 120 DAT (Fig. 6). At harvest, 3.1 and 1.31 mg·kg⁻¹ of Cd was found in the seeds of the RC and RBC rice, respectively (Fig. 6). This finding indicated that the bacterium reduced the accumulation of Cd in the seed by nearly 60% compared to that of the control (Fig. 6).

Cd translocation in the PL2 rice

The TF was calculated to define the transportation of Cd in the PL2 rice. The results showed that the bacterium had the ability to block the transport of Cd in the plant. Overall, the mobility of Cd in the RC shoots followed an upward trend (Fig. 7 RC). However, the Cd in the seed decreased, which represented a function of the crop to reduce the movement of Cd into the seed itself or was the result of the destruction of plant cells, which could not control the transport of the metal via the dysfunction of ion-exchange channels. Although the movement of Cd into the RBC rice was very high at 60 DAT, the TF continually decreased until harvest due to the presence of the bacteria (Fig. 7 RBC). At harvest, the transport of Cd into the PL2 seeds was similar in both treatments, but the activity of the bacteria in blocking the transfer of Cd into the shoots obviously differed from the control. According to the results, the amount of Cd in the RC seeds was more than double the

amount in the RBC seeds. Thus, the bacterium reduced the accumulation of Cd in the RBC rice seeds by nearly 60% (Fig. 6). This result indicated that the movement of Cd in the RBC seeds was a result of bacterial actions over the root functions. The possible mechanisms are summarized in Fig. 8.

Briefly, the Cd-tolerant bacteria coped with the presence of 5 μM CdCl_2 in the PL2 rice by binding and precipitating some of the Cd before it entered the root. Although some Cd and bacteria were absorbed by the plant, the bacterial cells acted as a biofilter to trap Cd and interrupt/reduce Cd transport to the aerial parts of the plant, which resulted in the enhancement of the growth of the whole plant and the yield components, especially the quantity and quality of the rice yield, and reduced the Cd stored in its grain. Although the Cd concentration in the RC rice was decreased at harvest similar to the RBC rice without the effects of the bacterium, the toxicity of Cd stress probably induced oxidative stress that destroyed the plant membrane and induced the leakage of various associated channels.

Discussion

The experiments in this study were performed via the inoculation of *C. taiwanensis* KKU2500-3 onto rice plants at the germination stage. The colonization of *C. taiwanensis* in the plant tissues and its effects on growth and the ability to cope with Cd toxicity were observed. Monitoring of the endophytic colonization of the plant by this bacteria was shown by SEM micrographs. The bacteria were able to grow and multiply in the rice plants with numbers that were effective for enhancing growth, improving seed production and reducing Cd absorption in rice. The bacteria were inoculated onto the rice seedlings at the germination stage (3 DAG); thus, the distribution of the bacteria into the roots and leaves of the rice began at the root surface and then may possibly have entered the roots via two approaches: 1) spreading through the base or cracks of root hairs or lateral root junctions

(Gopalaswamy et al. 2000; Gyaneshwar et al. 2001; 2002; James et al. 2002) or 2) entering upward through vascular tissue (xylem vessels) (Gyaneshwar et al. 2001; 2002). Our results revealed that the bacteria evidently moved from cell to cell through intercellular spaces, and some of the bacteria were located in the xylem. It was not surprising to find dense bacteria in the areas of the roots, stems and leaves that were not inoculated with the bacteria, results that are consistent with the observations reported by Gopalaswamy et al. (2000) and Mattos et al. (2008). Besides, this bacterial strain also improved rice productivity (in terms of both the quantity (grain yield) and quality (grain weight)) more than the control which is an important feature of plant growth-promoting bacteria used for biofertilizers, especially those with added nitrogen, which is the most important nutrient for rice production (Gyaneshwar 2002; Chaturvedi 2005). The KCU2500-3 strain not only exhibited this favorable feature but also supported rice growth in the presence of Cd stress (RBC), resulting in rice grains that could grow and produce grains similar to the control (Table 1).

In plants, Cd can be absorbed by the roots through other ion-exchange channels located in the cell membrane, including calcium (Clemens et al. 1998), iron (Cohen et al. 1998; Nakanishi et al. 2006), zinc (Cosio et al. 2004), magnesium, manganese, phosphorus and potassium channels (Sharma et al. 1985; Ding et al. 2012). After Cd enters a plant cell, it may disrupt many processes that inhibit cell proliferation; this effect is relative to the Cd concentration and exposure time. Unsurprisingly, the old leaves of some plants accumulate greater Cd levels than the younger leaves (Cataldo et al. 1981; McKenna et al. 1993). Moreover, more Cd was also found in the roots than in the shoots of some plants (Rauser 1995; Vázquez et al. 2006). The root cell wall has been suggested to be a tissue that accumulates a high concentration of Cd and is responsible to some extent for the Cd tolerance of the plants (Vázquez et al. 2006). Many tolerant plants can protect shoots from

excess Cd by storing more metal in the roots than can be stored by non-tolerant plants of the same species (Woolhouse 1983).

According to our findings, the Cd concentrations in the roots, shoots, and grains decreased. One reason for this finding is that rice plants have developed many approaches to cope with Cd, such as decreasing its bioavailability in the rhizosphere, chelating with phytochelatins, undergoing compartmentation in the cytosol (Cobbett and Goldsbrough 2002), retaining or eliminating the cell wall (Nishizono et al. 1987), and reducing the cellulose and lignin contents of the cell wall (Guo-sheng et al. 2007). In terms of phytoremediation, rice straw has the potential to remove Cd from the soil solution, since it accumulates high Cd levels in its roots and shoots. Ding et al. (2012) revealed that rice straw had a high Cd biosorption capacity. The main mechanisms were Cd ion exchange with other nutrient ions and chelation with various functional groups found in cellulose, hemicellulose, lignin and silica.

For some fraction of Cd, the available form of Cd in the soil solution and Cd uptake into plants are highly dependent on the pH, redox potential, presence of other ions, soil adsorption strength and other plant effectors that induce chelation. Similar to our findings, Cd has been reported to inhibit plant growth by producing active oxygen free radicals, disturbing ion homeostasis, and reducing the chlorophyll content and plant biomass (Sanità di Toppi and Gabbrielli 1999). In addition, this study also revealed that KKU2500-3 can recover the chlorophyll destroyed by Cd in RBC rice. The cultivation of rice in a nutrient solution containing Cd in a hydroponic system was used to control the bioavailable form of Cd based on the nutrient pH. Therefore, the plants easily absorbed total Cd. In contrast, total Cd in real conditions is not represented by the total soluble form of Cd and thus may not be absorbed by the crops. Growing rice using the hydroponic method revealed the mechanism

underlying the ability of the bacteria and rice to uptake and cope with Cd. Therefore, changing the rice cultivar may change the response mechanisms (Yu et al. 2006).

The most toxic symptoms of Cd poisoning are stunting and chlorosis. Stunting may be caused by inhibition of cell division and chromosomal abnormalities, whereas chlorosis may appear as a result of Fe deficiency or due to a direct/indirect interaction with iron (Fe) and the Fe:zinc (Zn) ratio in leaves (Garty et al. 1992), via chloroplast destruction (Ouzounidou et al. 1997) or through reaction with enzymes involved in the chlorophyll synthesis pathway (Böddi et al. 1995). Similar to our results, Cd has been reported to obstruct the creation of lateral roots in maize by turning the roots brown, rigid, and twisted. Cd also reduced coleoptile expansion and was accompanied by chlorosis and necrosis (Gunse et al. 1992; Wójcik and Tukendorf 1999; Nocito et al. 2002). Moreover, toxic Cd inhibits root mitosis in herbaceous plants by reducing the synthesis of cell wall constituents, destroying the Golgi body, and altering polysaccharide metabolism (Punz and Sieghardt 1993). Corresponding to our findings that Cd inhibited tiller formation in rice, Dong-Fen et al. (2008) demonstrated that the effects of Cd on rice growth and development mainly occurred during the early growth period by significantly inhibiting the tillering of the Cd-susceptible genotypes, which resulted in the reduction of dry matter accumulation during the entire growth period. Additionally, the grain yield was reduced. The main reason for the decrease in the grain yield in the Cd-susceptible genotypes was the effect on the reduction of the panicles and the spikelets per panicle under Cd stress.

Although the morpho-physiology of rice, especially its fibrous root system with a vast surface area for mineral absorption, favors Cd uptake (Coudert et al. 2010), the patterns of the rice plant responses to Cd were completely different between RC and RBC rice. While RC rice was destroyed by Cd (based on the growth disruption, pigment destruction, unhealthy characteristics and low yield), RBC rice growth was normal, despite the same

conditions. This result was the consequence of KCU2500-3 activity, suggesting a strategy to reduce Cd accumulation in rice plants. This strategy may include the Cd adsorption capacity of the bacteria and their ability to transform toxic Cd to the non-toxic form (Vig et al. 2003; Siripornadulsil and Siripornadulsil 2013). Furthermore, KCU2500-3 enhanced the production of some antioxidant enzymes in rice plants under Cd stress, which also may play a role in the response of rice plants to Cd toxicity (unpublished data). This result agreed with the observed Cd translocation from the roots to shoots and seeds of the plants. The non-bacterium inoculated rice showed a much higher TF than the bacteria-colonized rice. This TF value indicated the plants had a significant ability to cope with heavy metals to achieve phytoremediation (Yoon et al. 2006; Rezvani Zaefarian 2011). Comparison of the two treatments showed that the rice plants could exclude toxic metals outside the roots because the TF was lower than 1 (RC treatment), whereas the RBC treatment resulted in high performance of bacterial strain KCU2500-3 for enhancing the ability of rice plants to reduce Cd accumulation and the translocation of Cd to rice seeds. The plants with a TF lower than 1 were classified as Cd excluders (Rezvani and Zaefarian 2011). This finding leads to the concept of Cd-excluding rice, which not only tolerates high concentrations of Cd but also limits the entry of Cd into rice grains (Yu et al. 2006; Zhan et al. 2012). How rice plants and KCU2500-3 interact and play synergistic roles is yet to be elucidated.

The widespread association of *C. taiwanensis* with invasive *Mimosa* legumes has been reported (Andrus et al. 2012). These plants are common weeds and have spread throughout Thailand. *C. taiwanensis* KCU2500-3 was isolated from Cd-contaminated soil and endophytically colonized in rice plants. The rice growth-promoting properties of KCU2500-3 was observed, including the nitrogen-fixing and NH_3 production abilities (Punjee 2016). Such properties may be related to their synergistic action in rice under Cd stress. To our knowledge, the free-living KCU2500-3 does not form nodules but obviously enhanced root

hair formation in rice (Punjee 2016). Whether this strain can generate nodules in *Mimosa* spp. and carry *nodA* and *nifH*, the species versatile genes (Andrus et al. 2012), should be further investigated.

The concept of planting pollution-safe cultivars or low Cd-accumulating varieties in contaminated areas to accumulate the pollutant in their grains at a sufficiently low level for safe consumption has been previously proposed (Yu et al. 2006; Sriprachote et al. 2012a; 2012b). Based on the lower Cd content in the grain, these authors suggested that alternative varieties of rice should be grown in Cd-contaminated areas. Our results suggest that PL2 rice can adapt and grow effectively when colonized with *C. taiwanensis* KKU2500-3, yielding a Cd accumulation level 50% lower than that of the control, due to Cd exclusion. An approach involving the replanting of PL2 rice with colonizing bacteria in fields is proposed and recommended for farmers growing rice in Cd-contaminated areas.

Conclusion

Colonization of rice plants by *C. taiwanensis* KKU2500-3 via inoculating the bacteria into rice seedlings 3 DAG and before transplanting was highly effective and represented a low-cost technique. The maximum numbers of Cd-tolerant bacteria that colonized the RB and RBC plants grown in hydroponic systems were 9.03 and 9.60 log CFU·g⁻¹ plant, respectively. SEM analysis found attached bacteria on the root surfaces and internally colonized bacteria in the vascular tissues, cell walls and intercellular spaces of the enclosed cells. The colonizing bacteria enhanced the growth of the rice plant, particularly the growth of the root, as well as the yield components and grain yield, leading to a significant increase in the number and grain weight of the PL2 rice.

Although the Cd contents in the roots were very high (189.10 and 79.49 mg·kg⁻¹ for RC and RBC, respectively), the bacteria were able to decrease Cd accumulation by only

3.10 and 1.31 mg·kg⁻¹ in their grains, which demonstrated that the bacteria reduced Cd by 57.74% of the control content. The pattern of Cd reduction and the TF values of Cd in this crop indicated that the bacterium acted as an inhibitor of Cd translocation in the PL2 plants. These findings reveal the effectiveness of *C. taiwanensis* KKU2500-3 in the control of Cd translocation in rice plants. This phenomenon enables the development and application of this strain to diminish Cd accumulation in Cd-contaminated crops or to intensify Cd in hyperaccumulator plants, which is another attractive choice for phytoremediation.

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Conflicts of interest

The authors have no conflict of interest to declare.

References

- Andrus, A.D., Andam, C., and Parker, M.A. 2012. American origin of *Cupriavidus* bacteria associated with invasive *Mimosa* legumes in the Philippines. *FEMS Microbiol Eco.* 80: 747-750.

- Arwidsson, Z., Johansson, E., von Kronhelm, T., Allard, B., and van Hees, P. 2010. Remediation of metal contaminated soil by organic metabolites from fungi production of organic acids. *Water Air Soil Pollut.* 205: 215–226.
- Belimov, A.A., Hontzeas, N., Safronova, V.I., Demchinskaya, S.V., Puluzza, G. Bullitta, S., and Glick, B.R. 2005. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). *Soil Biology & Biochemistry* **37**: 241-250.
- Böddi, B., Oravecz, A.R., and Lehoczki, E. 1995. Effect of cadmium on organization and photoreduction of protochlorophyllide in dark-grown leaves and etioplast inner membrane preparations of wheat. *Photosynthetica* **31**: 411–420.
- Cataldo, D.A., Garland, T.R., and Wildung, R.E. 1981. Cadmium distribution and chemical fate in soybean plants. *Plant Physiology* **68**: 835-839.
- Chaturvedi, D. 2005. Effect of nitrogen fertilizers on growth, yield and quality of hybrid Rice (*Oryza sativa*). *Journal of European Agriculture* **6**(4): 611-618.
- Chen, W.M., Laevens, S., Lee, T.M., Coenye, T., De Vos, P., Mergeay, M. and Vandamme, P. 2001. *Ralstonia taiwanensis* sp. nov., isolated from root nodules of *Mimosa* species and sputum of a cystic fibrosis patient. *International Journal of Systematic and Evolutionary Microbiology* **51**: 1729–1735.
- Chen, W.M., James, E.K., Prescott, A.R., Kierans, M., and Sprent, J.I. 2003. Nodulation of *Mimosa* spp. by the β -Proteobacterium *Ralstonia taiwanensis*. *Molecular Plant-Microbe Interactions*. **16**(12): 1051–1061.
- Chen, W.M., James, E.K., Chou, J.H., Sheu, S.Y., Yang, S.Z., and Sprent, J.I. 2005. β -Rhizobia from *Mimosa pigra*, a newly discovered invasive plant in Taiwan. *New Phytologist* **168**: 661–675.

- Chen, W.M., Wu, C.H., James, E.K., and Chang, J.S. 2008. Metal biosorption capability of *Cupriavidus taiwanensis* and its effects on heavy metal removal by nodulated *Mimosa pudica*. *Journal of Hazardous Materials* **151**: 364–371.
- Chien, C.C, Wang, L.J., and Lin, W.R. 2014. Polyhydroxybutyrate accumulation by a cadmium-resistant strain of *Cupriavidus taiwanensis*. *Journal of the Taiwan Institute of Chemical Engineers* **45**: 1164–1169.
- Clemens, S., Antosiewicz, D.M., Ward, J.M., Schachtman, D.P., and Schroeder, J.I. 1998. The plant cDNA LCT1 mediates the uptake of calcium and cadmium in yeast. *Proc. Natl. Acad. Sci.* **95**: 12043-12048.
- Cobbett, C., and Goldsbrough, P. 2002. Phytochelation and metallothioneins: Roles in heavy metal detoxification and homeostasis. *Annu. Rev. Plant Biol.* **53**: 159-182.
- Cohen, C. K., Fox, T. C., Garvin, D. F., and Kochian, L.V. 1998. The role of iron-deficiency stress responses in stimulating heavy-metal transport in plants. *Plant Physiol.* **116**: 1063-1072.
- Codex Alimentarius Commission 2006: Citing online sources: Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission Report of the 29th Session. Available from [www.codexalimentarius.net/web/archives.jsp? year=04](http://www.codexalimentarius.net/web/archives.jsp?year=04) [accessed 31 May 2010].
- Cosio, C., Martinoia, E., and Keller, C. 2004. Hyperaccumulation of cadmium and zinc in *Thlaspi caerulescens* and *Arabidopsis halleri* at the leaf cellular level. *Plant Physiol.* **134**: 716-725.
- Coudert, Y., Périn, C., Courtois, B., Khong, N.G., and Ganet, P. 2010. Genetic control of root development in rice, the model cereal. *Trends Plant Sci* **15**(4):219–226.

- Dimkpa, C.O., Merten, D., Svatoš, A., Büchel, G., and Kothe, E. 2009. Metal-induced oxidative stress impacting plant growth in contaminated soil is alleviated by microbial siderophores. *Soil Biol. Biochem.* **41**: 154–162.
- Ding, Y., Jing, D., Gong, H., Zhou, L., and Yang, X. 2012. Biosorption of aquatic cadmium (II) by unmodified rice straw. *Bioresource Technol.* **114**: 20–25.
- Dong-Fen, H., Ling-Lin, X., Li-Nian, Y., Zhi-Qin, W., and Jian-Chang, Y. 2008. Comparison of agronomic and physiological traits of rice genotypes differing in cadmium-tolerance. *Acta Agronomica Sinica.* **34**(5): 809–817.
- Elliott, G.N., Chen, W.M., Chou, J.H., Wang, H.C., Sheu, S.Y., Perin, L., Reis, V.M., Moulin, L., Simon, M.F., Bontemps, C., Sutherland, J.M., Bessi, R., de Faria, S.M., Trinick, M.J., Prescott, A.R., Sprent, J.I., and James, E.K. 2007. *Burkholderia phymatum* is a highly effective nitrogen-fixing symbiont of *Mimosa* spp. and fixes nitrogen ex planta. *New Phytologist.* **173**: 168–180.
- European Union. 2006. European Commission Decision of 12 October 2006 amending, for the purposes of adapting to technical progress, the Annex to Directive 2002/95/EC of the European Parliament and of the Council as regards exemptions for applications of lead and cadmium (notified under document number C(2006) 4790)". *Official Journal of the European Union*: 14 October 2006.
- Farooq, U., Kozinski, J.A., Khan, M.A., and Athar, M. 2010. Biosorption of heavy ions using wheat based biosorbents—a review of the recent literature. *Bioresource Technol.* **101**: 5043–5053.
- Fayiga, A.Q., and Ma, L.Q., 2006. Using phosphate rock to immobilize metals in soils and increase arsenic uptake in *Pteris vittata*. *Sci. Total Environ.* **359**: 17–25.

- Garty, J., Karay, Y., and Harel, J. 1992. Effect of low pH heavy metals and anions on chlorophyll degradation in the lichen *Ramalina duriaei* (de not) Bagl. Environ. Experimen. Botany **32**: 229-241.
- Gonzalez-Chavez, M.C., Carrillo-Gonzalez, R., Wright, S.F., and Nichols, K.A., 2004. The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ. Pollu. **130**: 317–323.
- Gopalaswamy, G. Kannaiyan, S., O'Callaghan, K.J., Davey, M.R., and Cocking, E.C. 2000. The xylem of rice (*Oryza sativa*) is colonized by *Azorhizobium caulinodans*. Proc. R. Soc. Lond. B **267**: 103-107.
- Gu, J.G., and Zhou, Q.X. 2002. Cleaning up through phytoremediation: a review of Cd contaminated soils. Ecol. Sci. **21**:352–356.
- Gunse, B., Llugany, M., Poschenrieder, C., and Barcelo, J. 1992. Growth, cell wall elasticity and plasticity in *Zea mays* L. coleoptiles exposed to cadmium. Suelo y Planta **2**: 485–493.
- Guo-sheng, S., Ming-xue, C., Xiu-fu, Z., Chun-mei, X., Dang-ying, W., Qian, Q., and Guo-ping, Z. 2007. Cadmium Accumulation and Its Toxicity in Brittle Culm 1 (bc1), a Fragile Rice Mutant. Rice Science **14**(3): 217-222.
- Gyaneshwar, P., James, E.K., Mathan, N., Reddy, P.M., Reinhold-Hurek, and Ladha, J.K. 2001. Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. Journal of bacteriology **183**(8): 2634-2645.
- Gyaneshwar, P., James, E.K., Reddy, P.M., and Ladha, J.K. 2002. *Herbaspirillum* colonization increases growth and nitrogen accumulation in aluminium-tolerant rice varieties. New Phytologist **154**: 131–145.
- Hoagland, D.R., and Arnon, D.I. 1950. The water-culture method for growing plants without soil. California Agri. Exp. Station Cir. **374**: 1-32.

- Ishihara, T., Kobayashi, E., Okubo, Y., Suwazono, Y., Kido, T., Nishijyo, M., Nakagawa, H., and Nogawa, K. 2001. Association between cadmium concentration in rice and mortality in the Jinzu River basin. *Japan Toxicol.* **163**: 23–28.
- James, E.K., Gyaneshwar, P., Mathan, N., Barraquio, W.L., Reddy, P.M., Reddy, P.M., Olivares, F.L. and Ladha, J.K. 2002. Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. *Molecular Plant-Microbe Interactions* **15**(9): 894–906.
- Joshi, P.M., and Juwarkar, A.A. 2009. *In vivo* studies to elucidate the role of extracellular polymeric substances from *Azotobacter* in immobilization of heavy metals. *Environ. Sci. Technol.* **43**: 5884–5889.
- Juwarkar, A.A., Nair, A., Dubey, K.V., Singh, S.K., and Devotta, S. 2007. Biosurfactant technology for remediation of cadmium and lead contaminated soils. *Chemosphere* **10**: 1996–2002.
- Klonowska, A., Chaintreuil, C., Tisseyre, P., Miche', L., Melkonian, R., Ducousso, M., Laguerre, G., Brunel, B., and Moulin, L. 2012. Biodiversity of *Mimosa pudica* rhizobial symbionts (*Cupriavidus taiwanensis*, *Rhizobium mesoamericanum*) in New Caledonia and their adaptation to heavy metal-rich soils. *FEMS Microbiol Ecol* **81**: 618–635.
- Lichtenthaler, H.K., and Buschmann, C. 2002. Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. *Cur. Protocols Food Anal. Chem.* F4.3.1-F4.3.8.
- Lockwood, M.P. 1976. *Effects of Pollutants on Aquatic Organisms*. Cambridge University Press, New York.

- Lorestani, B., Cheraghi, M. and Yousefi, N. 2011. Accumulation of Pb, Fe, Mn, Cu and Zn in plants and choice of Hyperaccumulator plant in the industrial town of Vian, Iran. Arch. Biol. Sci., Belgrade, **63** (3), 739-745.
- Madhaiyan, M., Poonguzhali, S., and Sa, T. 2007. Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (*Lycopersicon esculentum* L.). Chemosphere **69**: 220–228.
- Marques, A.P.G.C., Moreira, H., Franco, A.R., Rangel, A.O.S.S., and Castro, P.M.L. 2013. Inoculating *Helianthus annuus* (sunflower) grown in zinc and cadmium contaminated soils with plant growth promoting bacteria – Effects on phytoremediation strategies. Chemosphere **92**: 74–83.
- McKenna, I.M., Chaney, R.L., and Williams, F.M. 1993. The effects of cadmium and zinc interactions on the accumulation and tissue distribution of zinc and cadmium in lettuce and spinach. Environ. Pollu. **79**: 113-120.
- Nakanishi, H., Ogawa, I., Ishimaru, Y., Mori, S., and Nishizawa, N.K. 2006. Iron deficiency enhances cadmium uptake and translocation mediated by the Fe²⁺ transporters OsIRT1 and OsIRT2 in rice. Soil Sci. Plant Nut. **52**: 464-469.
- Nishizono, H., Ichilawa, H., Suzuki, S., and Ishii, F. 1987. The role of the root cell wall in the heavy metal tolerance of *Athyrium yokoscense*. Plant Soil **101**: 15-20.
- Nocito, F., Pirovano, L., Cocucci, M., and Sacchi, G.A. 2002. Cadmium-induced sulphate uptake in maize roots. Plant Physiol. **129**: 1872–1879.
- Nogawa, K., Kobayashi, E., Okubo, Y., and Suwazono, Y. 2004. Environmental cadmium exposure, adverse effects and preventive measures in Japan. Biometals **17**: 581–587.
- Ouzounidou, G., Moustakas, M., Eleftheriou, E.P. 1997. Physiological and ultrastructural effects of cadmium on wheat (*Triticum aestivum* L.) leaves. Arch Environ Contam Toxicol **32**: 154–160.

- Platero, R., James, E.K., Rios, C., Iriarte, A., Sandes, L., Zabaleta, M., Battistoni, F., and Fabiano, E. 2016. Novel *Cupriavidus* strains isolated from root nodules of native Uruguayan *Mimosa* species. *Appl. Environ. Microbiol.* **82**(11): 3150-3164.
- Pluemphuak, T., Mala, T., and Kum Lung, A. 2014. Cadmium contents in rice grown in Cd contaminated paddy fields in Mae Tao floodplains Tak Province Thailand. *J. Science Tech.* **3**(2): 26-38.
- Punjee, P. 2016. Effects of cadmium-resistant bacteria on rice plant growth and its cadmium accumulation. Ph.D. Thesis, Khon Kaen University, Khon Kaen, Thailand.
- Punz, W. F., and Sieghardt, H. 1993. The response of roots of herbaceous plant species to heavy metals. *Environ. Exp. Botany* **33**: 85–98.
- Qi, B.C., and Aldrich, C. 2008. Biosorption of heavy metals from aqueous solutions with tobacco dust. *Bioresource Tech.* **99** (13): 5595–5601.
- Rajkumar, M., Sandhya, S., Prasad, M.N.V., and Freitas, H. 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. *Biotech. Advances* **30**: 1562–1574.
- Rauser, W.E., 1995. Retention of Cadmium in Root of Maize Seedlings. *Plant Physiol.* **109**: 195-202.
- Rezvani, M. and Zaefarian, M. 2011. Bioaccumulation and translocation factors of cadmium and lead in *Aeluropus littoralis*. *Aust. J. Agric. Engin.* **2**(4):114-119.
- Rocha, C.G., Zaia, D.A.M., Alfaya, R.V.D.S., and Alfaya, A.A.D.S. 2009. Use of rice straw as biosorbent for removal of Cu(II), Zn(II), Cd(II) and Hg(II) ions in industrial effluents. *J. Hazard. Mater.* **166**: 383–388.
- Sanità di Toppi, L., and Gabbrielli, R. 1999. Response to cadmium in higher plants. *Environ. Exp. Bot.* **41**: 105-130.

- Sharma, A., Mukherjee, A., and Talukder, G. 1985. Modification of cadmium toxicity in biological systems by other metals. *Cur. Sci.* **54**: 539-549.
- Sheng, X., He, L., Wang, Q., Ye, H., and Jiang, C. 2008. Effects of inoculation of biosurfactant-producing *Bacillus* sp. J119 on plant growth and cadmium uptake in a cadmium-amended soil. *J. Hazard Mater.* **155**: 17–22.
- Shi, J.Y., Lin, H.R., Yuan, X.F., Chen, X.C., Shen, C.F., and Chen, Y.X. 2011. Enhancement of copper availability and microbial community changes in rice rhizospheres affected by sulfur. *Molecules* **16**: 1409–1417.
- Simmons, R.W., Pongsakul, P., Saiyasitpanich, D., and Klinphoklap, S. 2005. Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice grain downstream of a zinc mineralized area in Thailand: Implication for public health. *Environ. Geochem. Health* **27**: 501-511.
- Siripornadulsil, S., and Siripornadulsil, W. 2013. Cadmium-tolerant bacteria reduce the uptake of cadmium in rice: Potential for microbial bioremediation. *Ecotoxicol. Environ. Safety* **94**: 94–103.
- Sriprachote, A., Kanyawongha, P., Ochiai, K., and Matoh, T. 2012a. Current situation of cadmium-polluted paddy soil, rice and soybean in the Mae Sot District, Tak Province, Thailand. *Soil Sci. Plant Nutrition* **58**(3): 349-359.
- Sriprachote, A., Kanyawongha, P., Pantuwan, G., Ochiai, K., and Matoh, T. 2012b. Evaluation of Thai rice cultivars with low-grain cadmium. *Soil Sci. Plant Nutrition* **58**(5), 568-572.
- Tripathi, M., Munot, H.P., Shouche, Y, Meyer, J.M., and Goel, R. 2005. Isolation and functional characterization of siderophore-producing lead- and cadmium-resistant *Pseudomonas putida* KNP9. *Curr. Microbiol.* **50**: 233–237.

- Vázquez, S., Goldsbrough, P., and Carpena, R.O. 2006. Assessing the relative contributions of phytochelatins and the cell wall to cadmium resistance in white lupin. *Physiol. Plant* **128**: 487-495.
- Venkatesh, N.M., and Vedaraman, N. 2012. Remediation of soil contaminated with copper using rhamnolipids produced from *Pseudomonas aeruginosa* MTCC 2297 using waste frying rice bran oil. *Ann. Microbiol.* **62**: 85–91.
- Vig, K., Megharaj, M., Sethunathan, N., and Naidu, R. 2003. Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review. *Adv. Environ Res.* **8**:121–135.
- von Rozycki, T., and Nies, D.H. 2009. *Cupriavidus metallidurans*: evolution of a metal-resistant bacterium. *Antonie van Leeuwenhoek* **96**(2):115-139.
- Wani, P.A., Khan, M.S., and Zaidi, A. 2007. Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp. (vigna) on growth, symbiosis, seed yield and metal uptake by greengram plants. *Chemosphere* **70**: 36–45.
- Wojcik, M., and Tukendorf, A. 1999. Cd-tolerance of maize, rye and wheat seedlings. *Acta Physiol. Plant.* **21**: 99–107.
- Woolhouse, H.W. 1983. Toxicity and tolerance in the response of plants. *In Encyclopedia of Plant Physiology. Edited by A. Pirson and M.H. Zimmermann.* Springer-Verlag, Berlin. New Series, Vol. 12C, pp. 245-300.
- Yanagisawa, M., Shinmura, Y., Yamada, N., Segawa, A., and Kida, K. 1984. Heavy metal pollution and methods of restoration of polluted soils in the Jinzu River basin. *Bull. Toyama Agric. Exp. Stn.* **15**: 101-110.
- Yang, Q., Tu, S., Wang, G., Liao, X., and Yan, X. 2012. Effectiveness of applying arsenate reducing bacteria to enhance arsenic removal from polluted soils by *Pteris vittata* L. *Int. J. Phytorem.* **14**: 89–99.

- Yoon, J., Cao, X., Zhou, Q., and Ma, L.Q. 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Science of the Total Environment* **368**: 456–464.
- Yu, H., Wang, J., Fang, W., Yuan, J., and Yang, Z. 2006. Cadmium accumulation in different rice cultivars and screening for pollution-safe cultivars of rice. *Sci. Total Environ.* **370**: 302–309.
- Zhang, W.H., Cai, Y., Tu, C., and Ma, Q.L. 2002. Arsenic speciation and distribution in an arsenic hyperaccumulating plant. *Sci Environ.* **300**: 167–177.
- Zwicker, R., Promsawad, A., and Zwicker, B. M. 2010. Cadmium content of commercial and contaminated rice, *Oryza sativa*, in Thailand and potential health implications. *Bull Environ. Contam. Toxicol.* **84**: 285–288.

Figure legends

Fig. 1. Number of Cd-tolerant bacteria predicted to be *C. taiwanensis* KKU2500-3 isolated from rice seedlings at 30-120 DAT in the absence (RB) and presence of 5 μM CdCl_2 (RBC).

Fig. 2. Electron micrographs of bacteria that are likely to be *C. taiwanensis* KKU2500-3 (arrows) colonizing the root surfaces and root interior of PL2 rice in the control (R), bacterium-inoculated (RB), Cd-supplemented (RC) and bacterium-inoculated and Cd-supplemented (RBC) treatments. h = root hair, x = xylem.

Fig. 3. Electron micrographs of bacteria likely to be *C. taiwanensis* KKU2500-3 (arrows) colonizing the interior of the shoot base and shoot tips of PL2 rice in the control (R), bacterium-inoculated (RB), Cd-supplemented (RC) and bacterium-inoculated and Cd-supplemented (RBC) treatments. h = root hair, x = xylem.

Fig. 4. Growth of PL2 rice (mean shoot and root lengths and shoot and root dry weights $\pm$ SD, $n = 3$) at 30-60 DAT after being grown in a hydroponic system using Hoagland's solution, under 4 treatments. Different letters indicate a significant difference at $p < 0.05$ (DMRT) for each parameter. The absence of a letter indicates that there is no significant difference between the treatments.

Fig. 5. Pigment contents (mean chlorophyll a, chlorophyll b and carotenoid contents $\pm$ SD, $n = 3$) of PL2 rice grown in a hydroponic system under 4 treatments. Different letters indicate a significant difference at $p < 0.05$ (DMRT) for each parameter. The absence of a letter indicates that there is no significant difference between the treatments at each DAT.

Fig. 6. Cd contents ($\pm$ SD, $n = 3$) of the roots, shoots and seeds of PL2 rice treated with 5 μM CdCl_2 (RC), inoculated with bacteria and treated with CdCl_2 (RBC). Different letters indicate significant differences at $p < 0.05$ (DMRT) for each DAT. * indicates significant differences between treatments at $p < 0.05$ (T-test).

Fig. 7. Translocation factor (TF) of Cd in PL2 rice grown in a hydroponic system under the RC and RBC treatments.

Fig. 8. A model of the possible roles of Cd-tolerant bacteria in the regulation of Cd translocation and the growth and yield of PL2 rice.

Figure 1

433x287mm (300 x 300 DPI)

Figure 2

281x388mm (300 x 300 DPI)

Figure 3

278x389mm (300 x 300 DPI)

Figure 4

278x297mm (300 x 300 DPI)

Figure 5

285x252mm (300 x 300 DPI)

Figure 6

264x165mm (300 x 300 DPI)

Figure 7

409x214mm (300 x 300 DPI)

Figure 8

266x136mm (300 x 300 DPI)

Table 1. Yield components and grain yield (mean $\pm$ SD, $n = 3$) of the PL2 rice.

Treatments	No. of tillers/plant	Panicle length (cm)	No. of grains/panicle	No. of filled grains/panicle	Filled grain percentage	100 grain weight (g)
R	2.0 $\pm$ 0.5 b	19.0 $\pm$ 4.7 b	47.2 $\pm$ 13.2 bc	25.3 $\pm$ 0.6 b	50.4 $\pm$ 8.1 bc	2.0842 $\pm$ 0.1 ab
RB	2.8 $\pm$ 0.7 c	23.9 $\pm$ 3.4 c	55.5 $\pm$ 17.3 c	33.7 $\pm$ 4.0 c	56.5 $\pm$ 4.5 c	2.4294 $\pm$ 0.4 b
RC	1.2 $\pm$ 0.2 a	15.1 $\pm$ 4.4 a	23.3 $\pm$ 10.6 a	6.7 $\pm$ 4.2 a	8.6 $\pm$ 4.1 a	1.7163 $\pm$ 0.1 a
RBC	2.0 $\pm$ 0.4 b	19.7 $\pm$ 3.0 b	40.9 $\pm$ 15.6 b	18.7 $\pm$ 1.2 b	36.3 $\pm$ 3.7 b	2.1498 $\pm$ 0.3 ab
<i>p</i> -value	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.05$

Draft