Mitigation of cadmium toxicity in Thai rice cultivar (PSL2) using biofertilizer containing indigenous cadmium-resistant microbial consortia

Preeyaporn Koedrith (✉ preeyaporn.koe@mahidol.edu)

Mahidol University <https://orcid.org/0000-0003-0160-6904>

Ladda Seang-On

Mahidol University

Weeradej Meeinkuirt

Mahidol University

Seriwat Saminpanya

Srinakharinwirot University

Research Article

Keywords: Bioremediation, Biofertilizer, Cd-resistant microbial consortia, Phytotoxicity, Indigenous soil microorganisms, Thai rice cultivar

Posted Date: September 21st, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1320460/v2>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Biofertilizer as amendment has growing awareness, little attention has been paid to the bioremediation potential of indigenous heavy metal-resistant microbes, especially when isolated from long term polluted soil, as a bioinoculant in biofertilizer. They are type of versatile nutrient provider and soil conditioner that is cost competitive, highly efficient with nondisruptive detoxifying capability. Herein, we investigated the effect of biofertilizers containing indigenous cadmium (Cd)-resistant microbial consortia on rice growth and physiological response. The Thai rice cultivar PSL2 (*Oryza sativa* L.) was grown in Cd-enriched soils amended with 3% biofertilizer. The composition of the biofertilizers' bacterial community at different taxonomic levels was explored using 16S rRNA gene Illumina MiSeq sequencing. Upon Cd stress, the test biofertilizer had maximum mitigating effects as shown by suppressed photosynthetic pigment loss, modulated proline content and enzymatic antioxidants, thereby allowing increased plant dry biomass (up to 115% and 112% in shoots and roots, respectively) and reduced Cd bioaccumulation (up to 68% and 65% in shoots and roots, respectively), as compared to the non-amended control. These phenomena might be attributed to increased soil pH, EC, CEC and organic matter, as well as enriched beneficial detoxifiers, i.e., *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* in the biofertilizers. The test biofertilizer was effective in ameliorating Cd phytotoxicity by improving soil biophysicochemical traits to limit Cd bioavailability, along with adjusting physiological traits such as antioxidative defense. This study first demonstrated that incorporating indigenous Cd-resistant microbe derived-biofertilizer could restrict Cd contents and consequently enhance plant growth and tolerance in polluted soil.

Introduction

Heavy metal pollution of soils has been a concerning and persistent environmental problem worldwide. Canal irrigation water mixed together with untreated industrial and agricultural wastewater is considered a major source of soil pollution; thus, degrading agricultural land (Rafique et al. 2019; Shah et al. 2020). Releasing large quantities of untreated wastewater containing heavily toxic metals, i.e., Cd, As, Ni, Pb and Cr, and letting them transfer by irrigation to water bodies and soils, leads to toxicity and lower yields of unsafe crops with unsatisfactory quality (Din et al. 2020; Gill et al. 2016; Tanwir et al. 2015). Cadmium (Cd) contamination of soil from various potential sources including mining and smelting, sewage sludge in agriculture, and industrial releases constitutes a severe environmental issue because Cd is nonbiodegradable and a highly toxic element conferring deleterious impacts on the food chain and to human health (Khan 2005; Rizwan et al. 2017; Tchounwou et al. 2012). It exhibits extreme toxicity even at low concentration, owing to its high accumulation, mobility and persistency in living systems (Javed et al. 2019). Cellular Cd influx in plants is mediated through calcium transport protein channels or specific membrane transport proteins involved in ion transport across plasmalemma (Lux et al. 2011). In cereal crops, Cd has adverse effects on seed germination, growth, transpiration, anti-oxidative systems, photosynthetic rate, nitrogen assimilation and yield (Javed et al. 2019; Rizwan et al. 2017; Sarwar et al. 2015; Tanwir et al. 2015).

Tak Province in northwestern Thailand, is located in close proximity to the Padaeng zinc (Zn) mining site, having areas exhibiting excessive levels of Cd in agricultural soils where health and environmental problems have been identified (Simmons et al. 2003). This has raised strong concerns because the International Water Management Institute discovered significant Cd contamination in rice grains and paddy soils in this province (Simmons et al. 2005). These elevated Cd levels (ranging from 3.4 to 284.0 mg kg⁻¹ in agricultural areas) are remarkably higher relative to the European Community limit of 3 mg kg⁻¹, posing high risk to the environment and human health (Swaddiwudhipong et al. 2012). The augmented deposition of Cd in water bodies and agricultural soils threatens the health status of plants, animals and humans.

Using industrial residues to remediate heavy metal contaminated wastewater has recently received attention as it not only facilitates the remediation efficiency of contaminants, but also promotes the recycling of wastes. For instance, innovative carbonaceous nano-chlorapatites (CNClAPs) synthesized by the mixture of bamboo residues and chlorapatites under pyrolysis at 400 to 600°C were used to remove Pb (II) and tetracycline from wastewater. The higher the pyrolytic temperature is, the higher the removal efficacy becomes. Adsorption testing has revealed that the interaction of Pb (II) and tetracycline could facilitate removing these dual contaminants at low concentrations in wastewater using CNClAPs (Deng et al. 2019). Furthermore, using sodium lignin sulfonate (SLS), a byproduct of the pulping and papermaking industry as a dispersant to synthesize a novel and ecofriendly SLS stabilized nano-chlorapatite (SNClAP), could promote the removal efficiency of Pb (II) and Cd (II) from sediment (Deng et al. 2020). Regarding the remediation potential of SNClAP in Cd-polluted sediment, the succession of microbial communities after remediation, especially phosphate-solubilizing bacterium *Pseudomonas*, was investigated. In addition, changes in microbial enzyme activity of catalase and urease were highly affected by the stability and bioavailability of Cd. It not only shed light on a novel remediation tool for Cd-polluted sediment but also provided insights on the change of microenvironments related to the stability and bioavailability of Cd in sediment (Deng et al. 2020).

Many physicochemical methods have been widely used to reduce toxicity and recover polluted agricultural sites. Alternatively, one bioremediation method using bioadsorbents, i.e., microorganisms, to reduce Cd mobility in soils can be adopted to cope with metal pollution in soils. The adsorption/removal of environmental pollutants via soil microbial metabolic potential provides an economical and safe technique compared with other physicochemical methods. Bioremediation using indigenous heavy metal-resistant microorganisms conferring heavy metal removal and plant growth promoting potential would prove a promising choice for agricultural sustainability in metal contaminated soil (Govindasamy et al. 2011).

Biofertilizers, are usually formed by the (semi-) solid state fermentation of agro-industrial wastes, and consist of both beneficial microbes and primary nutrients or plant growth regulating substances (Chen et al. 2011). Incorporating biofertilizers in soil would help produce antibiotics and stimulate biodegradation of soil organic matter (OM), increasing nutrient supplies, and enhancing plant tolerance to environmental stress. Microbial strains isolated from polluted environments exhibited resistance to higher levels of

metals than those isolated from unpolluted areas (Rajkumar et al. 2010). Through metal-stress responsive mechanisms, soil microbes applied as biofertilizers effectively promoted the growth of plants implanted in heavy metal enriched soils by lowering metal-induced phytotoxicity (Madhaiyan et al. 2007;Wani &Khan 2010). In addition, other mechanisms, i.e., plant growth promoting bacteria (PGPB) boost plant development. For instance, they protect colonizing plants by suppressing pathogens by producing antibiotics, hydrogen cyanide and phenazines etc.(Cazorla et al. 2007;Saravanakumar et al. 2007). PGPB also enhance plant growth via N₂ fixation (Jha &Kumar 2007), solubilizing insoluble phosphorus (Ahemad &Khan 2012b), forming siderophores (Jahanian et al. 2012;Tian et al. 2009) and phytohormones (Ahemad &Khan 2012a;Ahemad &Khan 2012b;Tank &Saraf 2010), reducing ethylene content (Rodrigues et al. 2008;Tank &Saraf 2010), synthesizing antibiotics and antifungal metabolites and inducing systemic resistance (Glick 2012). Also, PGPB are able to increase the soil fertility and in turn, the plant yield by providing essential nutrients, growth regulating substances (Ahemad &Khan 2012d) and lowering ethylene-based stress by inducing 1-aminocyclopropane-1-carboxylate deaminase and facilitating plant resistance to abiotic contaminants, e.g., metals and pesticides (Ahemad &Khan 2012c;Glick 2012;Khan 2005). Exploiting the potential of PGPB to detoxify metals as well as versatile plant advantageous characteristics constitutes a potent, cost effective and ecofriendly metal bioremediating tool. Hence, biofertilizers have been recognized as clean and efficient soil conditioners or amendments to improve soil characteristics (Bhardwaj et al. 2014;Gajdos et al. 2012;Shen et al. 2013).

Nevertheless, the efficiency of biofertilizers derived from indigenous Cd-resistant microbial consortia isolated from Cd-contaminated soil in reducing Cd phytotoxicity has not been well documented. Accordingly, soil with long term Cd contamination caused by the mining and smelting activities in Tak Province of Thailand was selected as our focus. The overall objective of this study was to evaluate the effect of biofertilizer treatments on alleviating Cd phytotoxicity by assessing plant growth performance, physiological response and Cd bioaccumulation within different parts of the Thai rice cultivar PSL2 (*Oryza sativa* L.) until the physiological maturity growth stage. The effects of test biofertilizers on soil properties and bioavailable Cd content were also examined at growth end stage.

Materials And Methods

Collection and analysis of soil samples

The long term polluted top soils (<20 cm in depth) used for greenhouse experiments were sampled from an agricultural area in Pha Dei Village, Mae Sot District, Tak Province, Thailand (N 16° 40' 35.9" E 98° 37' 37.4") at an altitude of 197 m. The soil at this site was tilled for either rice-corn or rice-bean crops in one cropping year. The selected physicochemical characteristics of the soil are shown in Table 4-1 & 4-2. Soil samples were divided in two main portions: one for physicochemical characterizations and the other for enriched culture following biofertilizer preparation.

Soil material was homogenized, air-dried, crushed, and sieved (2-mm mesh size). The following physicochemical properties of the soil were determined: pH and electrical conductivity (EC) (1:5

soil/water suspensions) using a pH meter and an EC meter respectively; OM content by wet oxidation and titration according to the modified Walkley-Black procedure (Nelson & Sommers 1996); cation exchange capacity (CEC) using 1 N ammonium chloride pH 7.0 after pretreatment to remove soluble salts (Oorts et al. 2007); total N by the Kjeldahl method; extractable P by Bray II method (Bray & Kurtz 1945) and extractable K using an atomic absorption spectrophotometer (Perkin Elmer Analyst 200, USA) after ammonium acetate extraction at pH 7.

Preparation and analysis of Cd-resistant biofertilizer

The biofertilizers used as amendments for remediation of Cd contaminated soils were prepared using repeated culture enrichment of the soil Cd-resistant bacteria as previously described (Seang-On et al. 2019) followed by semi-solid fermentation/biofertilization conditions. Topsoil (<20 cm in depth) was collected from a long-term Cd and Zn-contaminated agricultural area in Pha Dei Village, Mae Sot District, Tak Province (N 16° 40' 35.9" E 98° 37' 37.4") at an altitude of 197 m for culture enrichment. To enrich Cd-resistant bacteria (BC), the first 5 g of each topsoil sample was added to 95 ml of nutrient broth (NB, 0.5% peptone, 0.3% meat extract, pH 7.0) containing 50 or 100 ppm Cd chloride (CdCl_2). After two weeks of consecutive incubation at 30°C, the bacteria were cultured on nutrient agar plates (NA, nutrient broth and 1.5% agar) supplemented with CdCl_2 for 72 h at 30°C. The colonies of Cd-resistant bacteria were quantified as colony forming units per ml (CFU ml^{-1}). The test biofertilizer (BF) was prepared under aerobic conditions, using enriched BC with rice bran supplemented with micronutrients and mineral additives to stimulate fermentation. The organic fertilizer (OF) was produced by fermenting the rice bran supplemented with micronutrients and mineral additives as mentioned in an aerobic environment, in absence of BC. The biofertilizers were stored at 4°C prior to use in greenhouse experiments. Hence, the treatments used in this study were listed in Table 1. The main components and bacterial compositions of each amendment are shown in Table 2.

For physicochemical analyses, properties of the biofertilizers as soil amendments or conditioners were determined: pH using a pH meter and OM content using wet oxidation and titration according to the modified Walkley-Black procedure (Nelson & Sommers 1996). Total contents of metal elements including Cd, Zn, Ca, Mg, S, Fe and Mn in biofertilizer samples were determined using microwave digestion and quantification using an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 200).

Bacterial diversity and composition of the test biofertilizers compared with the enriched consortia were analyzed using 16S rRNA gene Illumina MiSeq sequencing as previously described (Seang-On et al. 2019). Total genomic DNA was extracted from 10 ml of the enriched culture and the biofertilizers were tested (three biological replicates per treatment) using QIAamp® DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer instructions with some modifications. The 16S rDNA (V3-V4) bacterial primers containing the Illumina overhang adapter sequences (as underlined) 341F (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and 805R (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) were used for PCR amplification (Herlemann et al. 2011). The PCR mixtures (25 µl) contained 12.5 µl of 2x KAPA HiFi Hot

Start Readymix (KAPA Biosystems, USA), 5 μl of each primer (1 $\mu\text{mol l}^{-1}$) and 2.5 μl of target DNA (5 $\text{ng } \mu\text{l}^{-1}$). The PCR cycling conditions consisted of an initial denaturation step at 94°C (3 min), followed by 25 cycles of 98°C (20 sec), 55°C (30 sec) and 72°C (30 sec) and a final elongation at 72°C (5 min). The PCR products were cleaned-up on AMPure XP beads (Agencourt Bioscience, USA). The purified amplicons (550-bp fragments) were submitted to the Omics Sciences and Bioinformatics Center (Chulalongkorn University, Bangkok, Thailand) for paired-end sequencing on the Illumina MiSeq platform. Subsequently, the purified 16S RNA gene amplicons were then indexed using 2X KAPA hot-start ready mix and 5 μl of each Nextera XT index primer in a 50 μl PCR reaction, followed by 8 to 10 cycles of PCR amplification. The PCR cycling was set as aforementioned. Next, the indexed 16S RNA gene amplicons were purified on AMPure XP beads (Agencourt Bioscience, USA), pooled and diluted to a final loading concentration of 4 pM. Cluster generation and 250-bp paired-end read sequencing were performed on an Illumina MiSeq using the MiSeq Reagent Kit. Amplicon sequence analysis was performed with QIIME version 1.9.0. (Caporaso et al. 2010). All sequence reads were sorted based on their unique barcodes, trimmed for sequence quality and clustered at 97% identity for operational taxonomic units (OTUs). The UCHIME algorithm was used to discard chimera sequences (Edgar et al. 2011).

The microbial diversity index in terms of diversity (Shannon index) and richness (Chao1 index) were subsequently computed using MOTHUR (Schloss et al. 2009). To investigate the microbial composition and diversity, the Shannon diversity index, an estimator of species richness and diversity using a natural logarithm, accounts for both abundance and evenness of the taxa present, while the Chao1 richness estimator reflects diversity from abundance data and the number of rare taxa missed from under-sampling.

Greenhouse experimental design

All the experiments involving plants adhered to the relevant ethical guidelines on plant usage. Table 1 present the treatments used in this study. A 2,000 g soil sample was crushed, sieved (2-mm mesh size), and placed in a plastic pot as previously described with some modifications (Wang et al. 2019). Biofertilizers were mixed in long-term Cd contaminated soil at a rate of 3%. Hence, NPK basal fertilizer containing 0.25 g urea kg^{-1} soil, 0.15 g KH_2PO_4 kg^{-1} soil and 0.04 KCl kg^{-1} soil was initially dissolved in deionized water and thoroughly mixed with the soil in each pot. Subsequently, all pots were incubated with moisture at 75% of water holding capacity for five weeks to allow the nutrients in biofertilizers to be released in the soil, as well as promote the microbes in biofertilizers to work and to functionally act toward Cd stress. Thai rice seeds (PSL2) were sterilized with 5% hydrogen peroxide (H_2O_2) for 5 min, rinsed with distilled water and placed in a Petri Dish containing two pieces of filter paper. After germination, eight rice seedlings were transplanted in each plastic pot. The pots were arranged in a randomized complete block design with six replicates for each treatment. During rice growth, each pot was irrigated every three days with distilled water to maintain soil moisture at ca. 60 to 70% of water holding capacity. Greenhouse conditions were as follows: temperature 26 to 40°C, 55 to 70% relative humidity, 5,500 to 50,000 lx light intensity, and a 12/12 h photoperiod.

After 30 days of treatment, the roots and shoots were collected separately per biological replicate and stored at 4°C for measuring proline content, photosynthetic pigments and different enzymatic assays.

Four months after transplantation, the plant samples were washed with tap water, rinsed with deionized water several times until all excess soil was removed, and then the shoots and roots were harvested. Plant materials were oven-dried at 80°C for four days before determining weight. Soil material was collected from each pot and allowed to air-dry for five days. Soil and plant samples were subjected to chemical analyses.

Measurement of total protein content

The total protein content of rice leaves was quantified as previously described (Lowry & Rosebrough 1951). Plant leaves (0.5 g) were ground and added with phosphate buffer. The mixture was centrifuged at 3,000 rpm for 10 min. The resulting supernatant (0.1 mL) was added with distilled water to make the volume up to 1 mL. This solution was added with the equal volume of alkaline CuSO₄ reagent and shaken for 10 min. Finally, the Folin reagent was added and then incubated for 30 min at 28 ± 2 °C. Readings were measured at 650 nm. Bovine serum albumin (BSA) was taken as a reference for the calculation of total protein contents.

Estimation of photosynthetic pigments

Photosynthetic pigments (chlorophyll (Chl) a, b, and carotenoids) of rice leaves were estimated as previously mentioned (Burnison 1980). Plant leaves (0.5 g) were added with 10 ml of dimethyl sulfoxide (DMSO) and then heated at 65°C in water bath for 4 hrs. The supernatant was separated, and its absorbance was recorded at 663 nm, 645 nm, for Chl a, Chl b, and 480 nm for carotenoids, respectively.

Estimation of proline content

Proline contents were determined by using previous protocol (Bates et al. 1973). Rice leaves (0.5 g) were ground in 80% ethanol and then heated at 80°C for 1 hr in a water bath. After centrifugation, 0.5 ml supernatant was taken into a new test tube, added with 0.5 ml dH₂O and 1 ml of 5% phenol, and placed in an incubator for 1 hr. After incubation, 2.5 ml sulfuric acid was added and the readings were measured at 490 nm.

Determination of enzymatic antioxidant activities

Enzyme extracts

For preparing enzyme extracts, 0.5 g leaves and roots were ground in 3 ml phosphate buffer (pH 7.8) and subjected to homogenization on ice. The solution was made to 5 ml and centrifuged at 13,000 rpm for 20 min at 4°C. The supernatant was covered with aluminum foil to avoid light exposure and stored at 4°C for subsequent enzyme assays.

Ascorbate peroxidase (APX) activity

APX (EC# 1.11.1.11) activity was quantified by examining the rate of ascorbate oxidation at a wavelength of 290 nm as the previous method (Asada 1987). The reaction mixture consisted of 50 mM phosphate buffer pH 7.0, 0.1 mM H₂O₂, 0.5 mM ascorbic acid, and 100 µl of enzyme crude extract.

Superoxide dismutase (SOD) activity

SOD (EC# 1.15.1.1) activity was subjected to assess the inhibition in the photoreduction of nitro blue tetrazolium (NBT) as previous procedure (Beyer Jr & Fridovich 1987). Reaction mixture was taken with 50 mM sodium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 mM sodium carbonate, 12 mM L-methionine, 50 µM NBT, 10 µM riboflavin, and 100 µl of enzyme crude extract. For comparison, a set of reactions with all components except the crude extract was taken as control. To start the reactions, the reaction tubes were exposed to white light for 15 min. Reactions were terminated by switching off the lights and readings were recorded at 560 nm.

Sampling and metal analyses of soil and plant tissue

Total and extractable contents of metal elements in soil and plant samples were determined using microwave digestion and diethylenetriamine pentaacetate (DTPA) extraction, respectively. Total (strong acid-extractable) Cd and Zn in soil before and after planting was estimated by digesting approximately 1 g of air-dried soil with 4.5 ml of 37% hydrochloric acid (HCl), 1.5 ml of 65% nitric acid, and 1 ml of 30% H₂O₂ in a microwave digestion system (Milestone ETHOS One, USA). A similar procedure was employed to digest plant materials, but without HCl addition. The amount of DTPA-extractable Cd and Zn in soil was determined using 0.005M DTPA+0.01 M CaCl₂+0.1M triethanolamine, pH 7.30 at a soil-to-solution ratio (w/v) of 1:2. The metal concentrations in both the digests and extracts were quantified using an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 200).

Statistical analyses

Data were subjected to statistical analysis using two-way ANOVA (SPSS Software) to detect significant differences with 95% confidence level (P -value ≤ 0.05).

Results

Relative abundance and composition structure of the test biofertilizers

The relative abundance and composition of the test biofertilizers were assessed and compared with the enriched culture consortium using a 16S RNA gene amplicon sequencing approach. Table 2 summarizes bacterial composition in phyla of the biofertilizer. Table 3 shows the alteration in the diversity indices of the biofertilizers BF, compared with the enriched bacterial consortia BC. Diversity indices of the biofertilizers BF were significantly higher than those of the enriched bacterial consortia BC samples, as shown by the increase in the Shannon diversity index and the Chao1 richness estimator.

Fig. 1 shows the relative abundance of bacterial phyla in the biofertilizers and the enriched bacterial consortia. *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Gemmatimonadetes* were the most dominant phyla. Rising Cd concentrations (20 to 100 ppm) added in the microbial culture could enrich a population of Cd-resistant bacterial phyla among other Cd-sensitive phyla (Seang-On et al. 2019). These enriched population of Cd-resistant bacteria was inoculated into the test biofertilizer used in this study. Using 16S RNA gene amplicon sequencing, the three top main phyla including *Firmicutes*, *Bacteroidetes* and *Proteobacteria* were explored relative to the corresponding enriched culture (Table 3 and Fig. 1a). The relative abundance of *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* increased across the biofertilization process, while those of *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Planctomycetes* and *Verrucomicrobia* decreased. Although a decrease in *Acidobacteria*, *Gemmatimonadetes*, and *Planctomycetes* was observed, they still somewhat remained throughout the biofertilization process. In the biofertilizer, predominant detoxifiers at a finer taxonomic level of *Proteobacteria* (including *Comamonas* sp., *Pseudomonas* sp., *Stenotrophomonas* sp., *Acinetobacter* sp., and *Delftia* sp.), *Firmicutes* (including *Enterococcus* sp., *Lactobacillus* sp., and *Lactococcus* sp.), and *Bacteroidetes* (including *Wautersiella* sp., *Myroides* sp., *Cloacibacterium* sp., *Paludibacter* sp.) were explored among other genera (Fig. 1b). The biofertilizer containing indigenous Cd-resistant bacterial consortium were successfully prepared and subjected to subsequent investigation.

The effects of biofertilizer on soil physicochemical traits

Table 2 shows physicochemical properties and bacterial composition in phyla of biofertilizer BF in comparison to the organic fertilizer OF. Under Cd stress, the application of biofertilizer BF showed maximum increase in soil pH, EC, CEC, and OM as well as major mineral nutrition N, P, and K as compared with the non-amended control, with higher degree of increase than that of the other amendments (Table 4-1). This might be a result of the intrinsic pH of the biofertilizer (Table 2). The treatments by soil pH (Table 4), OM content (Table 2) and tissue Cd content (Table 6) were ranked in the order as BF > OF. All the amendments showed increase in soil pH and OM with different extent, these treatments could lower soil bioavailable Cd and rice tissue Cd contents as compared with the control (Table 4-1, 4-2 & 6).

The level of DTPA-extractable metals including Cd, Zn, Fe, Mg, and Mn could be recommended as an potential indicator of the pool metal availability in soils and consequent bioaccumulation in plants (Catherine et al. 2006;Sun et al. 2007). All the amendments showed decrease in the levels of DTPA-extractable Cd and Zn compared with the control (Table 4-2). Particularly, the application of biofertilizer exhibited the lowest level of DTPA-extractable Cd, as shown by a 54% decrease in the Cd level compared with the non-amended control. This point might imply that in this phenomenon, there was association between the soil physical structure or chemical composition/fertility of the biofertilizers and the Cd availability/stability in soils.

The effects of biofertilizer on rice growth under Cd stress

Plant growth is a plausible determinant that reflects Cd phytotoxicity in polluted soils. The plants showed less biomass after exposure to Cd as compared to the non-stressed control. Cd mitigating potential of

biofertilizer was observed for the rice plants grown in Cd-enriched soil over 4 months. Exposure of rice plants to Cd stress negatively affected the germination (as shown in our previous study, Seang-On et al. 2019) and their growth performance (Table 5).

All the amendments increased tendency of biomass and length of rice shoot and root under Cd stress, as compared to the non-amended stressed control CD (Table 5). Upon Cd toxicity, the application of biofertilizer showed maximum increase in shoot and root dry weight by 115% and 112% respectively, as compared to the control (Table 5). Results in Table 5 also displayed that all the amendments had alleviating potential on Cd stress in terms of shoot and root length. Especially, the biofertilizer caused a maximum effect by 61% increase in shoot length and 26% decrease in root length, as compared to the control.

Effect of biofertilizer on mineral nutrition and Cd bioaccumulation toward Cd stress

Due to the extent of stress and growth depending on nutrient uptake and translocation, Cd toxicity decreased N and P

content in rice shoots. Under Cd stress, all the amendments were able to maintain higher content N and P than the non-amended control (Table 6). Particularly, the biofertilizer amendment was effective in elevating maximum levels of shoot N and P, as compared with the other amendments in presence of Cd (Table 6). This indicated that the microbial consortium in biofertilizer play a role, in part, in mitigating Cd toxicity in rice plant.

Table 6 also reveals the effect of biofertilizer on tissue Cd content. The application of biofertilizer BF significantly reduced Cd contents in rice root and shoot ($p < 0.05$), implying that the test biofertilizer may effectively immobilize Cd in soils and impair its translocation to rice tissues. This decrease in Cd accumulation in plants after applying the biofertilizers could be due to improved soil physical properties and nutrient availability (Table 4).

Effect of biofertilizer on photosynthetic pigments in response to Cd stress

Cd toxicity may lead to a loss of photosynthetic pigments, resulting in chlorotic symptoms. Our results showed that Cd stress caused a remarkable decrease in chlorophyll (Chl) a, Chl b, and carotenoid contents whereas the application of biofertilizer effectively suppressed these losses of pigments in presence of Cd (Table 7). Under Cd stress, the biofertilizer showed a maximum increase in Chl a, Chl b, total Chl, and carotenoid synthesis by 150%, 125%, 144%, and 114% respectively, as compared to the non-amended stressed control.

The effect of biofertilizer on proline content and enzymatic antioxidants upon Cd stress

Cd stress disturbed the water balance, leading to osmolyte accumulation in the rice leaves, as indicated by a great increase in proline content, but all the amendments except organic fertilizer OF suppressed the accumulation of shoot proline in comparison to the control under Cd stress. Cd-stressed rice seedlings

showed a 271% increase in proline contents, as compared to the non-stressed control (Table 8). However, the biofertilizer amendment resulted in a 46% decrease in the proline synthesis, as compared to the non-amended stressed control.

Plants have vital strategies for avoidance of Cd toxicity. For the prime line on counteracting excessive ROS and serving as cellular redox buffers, the plant's antioxidant defense system consists of antioxidants that are enzymatic (e.g., superoxide dismutase SOD, ascorbate peroxidase APX, catalase CAT, and glutathione-S-transferase GST) and non-enzymatic (ascorbic acid, glutathione, tocopherol, and phenolic compounds) (Sharma et al. 2012). Both enzymatic and non-enzymatic antioxidants work simultaneously to combat the Cd-induced oxidative stress. Upon exposure to metal stress, the plant's enzymatic antioxidant activity gradually rises up with increasing metal concentration while these activities become dropped down, and enzymatic defense system is ultimately disrupted with too high metal concentration. Cd stress stimulated the activities of the antioxidant enzymes superoxide dismutase SOD and ascorbate peroxidase APX. The Cd-stressed plants showed 63%, 67% and 84%, 70% increase in SOD and APX activities of leaves and roots respectively, as compared to the non-stressed control seedling (Table 8). Under Cd stress, all the amendments except organic fertilizer OF further increased SOD and APX activity, as compared to the non-amended stressed control. In particular, application of biofertilizer BF was more effective in inducing SOD and APX activity both in roots and shoots than the other amendments. The biofertilizer amendment showed maximum SOD and APX activities by 171% and 155% in shoots and 114% and 110% in roots respectively, as compared to the control (Table 8). As noteworthy, the tested biofertilizer was effective in alleviating the Cd toxicity by enhancing activities of SOD and APX in rice shoots and roots.

Discussion

Among metals, Cd is recognized as highly toxic metal that impacts the growth and physiological processes particularly during early stages of growth and development of the plant, thereby leading to significant yield loss (Javed et al. 2019; Rizwan et al. 2017; Sarwar et al. 2015; Tanwir et al. 2015). Exposure of rice plants to Cd stress negatively affected the seed germination (Seang-On et al. 2019). To reduce toxicity and remediate polluted agricultural areas, soil amendments of biofertilizer containing bioagents and fertilizer are renowned with multi-modalities to enhance plant tolerance to environmental stress, nutrient supplies and biodegradation of organic matter (Chen et al. 2011). Indeed, indigenous microbial strains isolated from long-term polluted paddy site displayed resistance to Cd at higher levels than those isolated from unpolluted soil (Saeng-On et al. 2019). The efficiency of biofertilizers derived from indigenous Cd-resistant microbial consortia isolated from Cd-contaminated soil in mitigating Cd phytotoxicity has not been well elucidated yet.

Our study firstly demonstrated that the soil amendment of biofertilizer containing indigenous cadmium-resistant microbial consortia could mitigate the adverse effect of Cd and improve the plant growth by restoring photosynthetic pigment losses and supplying essential nutrients in Thai rice cultivar (PSL2). Additionally, the test biofertilizer was effective to overcome water imbalance and oxidative stress by

adjusting the proline content and the activities of antioxidant enzymes, respectively, and to limit Cd bioaccumulation by manipulating soil physicochemical traits.

Bacterial composition structure of the test biofertilizers

In the biofertilizers, *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Gemmatimonadetes* were determined as the most dominant bacterial phyla using 16S rRNA gene Illumina MiSeq sequencing (Fig. 1). In consistent findings with related studies, the biofertilizer pH and organic carbon could affect the abundance of *Bacteroidetes*, *Gemmatimonadetes* and *Proteobacteria*, and these phyla were also dominant in biofertilizer (Wang et al. 2019). It suggested that the biofertilizer pH and organic carbon played key roles in shaping the enriched microbial communities. Moreover, bioavailability soil Cd could positively influence the microbial communities in the biofertilizer-treated soils, and some Cd-coexistence bacteria, i.e., *Chloroflexi*, *Acidobacteria*, and *Saccharibacteria* might have become dominant due to excess Cd in rhizosphere soils (Wang et al. 2019). In addition, the solubility and availability of soil phosphate is determined by specific microbial activities, while soil phosphate concentration becomes a determinant for Cd phytotoxicity (Wang et al. 2019).

One promising method for alleviating Cd stress and promoting plant growth is the bioaugmentation of microorganisms. For instance, the amendment of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Cupriavidus taiwanensis* and *Beauveria bassiana* to soil significantly restricted tissue Cd content in rice (*Oryza sativa*) and improved plant growth performance under Cd stress, owing to the biotransformation of the toxic Cd form to nontoxic insoluble form of Cd sulfide (CdS) and adsorption by Cd-binding proteins (Siripornadulsil & Siripornadulsil 2013; Suksabye et al. 2016). Another related study showed that inoculating with plant growth-promoting rhizobacteria (PGPR) promoted maize growth and reduced shoot Cd contents compared with those of the untreated control (Moreira et al. 2014). Related findings revealed that adding Cd-resistant *Micrococcus* sp. TISTR2221 enhanced maize growth and retained Cd concentration in grains (Sangthong et al. 2016). Although soil microorganisms have been extensively used to promote plant performance and restrict soil Cd, directly adding microorganisms into fields together with an appropriate organic substrate is recommended to maintain their substantial activities over a long period (Shen et al. 2015). The combined advantages of bioagents and fertilizers would be an alternative to mitigate Cd phytotoxicity.

The effects of biofertilizer on soil physicochemical traits

Our study revealed that the application of the biofertilizer BF increased soil chemical properties such as pH, EC, CEC, and OM content (Table 4-1), in addition to decrease in DTPA-extractable Cd and Zn (Table 4-2). Mineral nutrition containing basic cations (K, Ca, and Mg) in the biofertilizer could contribute to the increase in soil pH, EC and CEC, while organic residues derived from agricultural wastes were involved in nourishment of soil organic carbon. Increase in soil EC and CEC could also be regarded as key factors in modulating heavy metal exchangeability and bioavailability (Abdelhafez et al. 2014; Lu et al. 2014). Increasing soil pH after applying biofertilizer could increase the negatively charged surfaces as well as alkaline conditions, such as hydroxide and carbonate groups, which might support active substances for

surface sorption, precipitation and complexation; thus, reducing heavy metal bioavailability with less possibility to enter the food chain (Younis et al. 2015).

Compared to the control, the application of biofertilizer BF remarkably increased soil pH, but the amendment OF had less impact on increasing soil pH (Table 4-1). This could be due to the initial pH of the amendments (Table 2). Although applying the amendment OF had slight effect on soil pH, this amendment still decreased Cd contents in rice tissues compared to the control (Tables 4 & 6). These results indicated that besides pH, the mineral nutrients, e.g., N, P, K, Ca, Mg, S, Fe, and microorganisms in the biofertilizer might play crucial roles in controlling Cd uptake and bioaccumulation (Catherine et al. 2006; Sun et al. 2007). Related studies reported that soil pH, DTPA-extractable Cd, total phosphorus and organic carbon were determined as the most pivotal environmental factors contributing the changes in microbial community composition (Wang et al. 2019). Additionally, soil organic carbon served as the carbon source to bacteria was considered crucial. Indeed, soil pH and OM are considered important factors for limiting Cd availability (Khan et al. 2017; Wang et al. 2019). Moreover, potential parameters other than soil pH and OM, including the mineral nutrition, e.g., N, P, K, Ca, Mg, S, Fe, and microorganisms in the biofertilizer itself remain effective in suppressing Cd bioaccumulation (Catherine et al. 2006; Sun et al. 2007).

The effects of biofertilizer on rice growth under Cd stress

The retarded plant growth toward Cd stress might be due to various factors, where it adversely affects water status, nutrient assimilation, mineral homeostasis, photosynthesis, oxidative stress response, etc. Our study indicated that the biofertilizer BF was effective in promoting growth performance to rice plant under Cd stress (Table 5). This may be elucidated by the fact that beneficial microorganisms as well as mineral nutrition in the biofertilizer can facilitate Cd immobilization in the soil and lower its toxicity to roots; possibly allowing plants to assimilate more nutrients (Nejad et al. 2018).

Effect of biofertilizer on mineral nutrition and Cd bioaccumulation toward Cd stress

Among the other amendments, the biofertilizer BF exhibited the lowest level of tissue Cd as shown by decrease in shoots and roots Cd contents 68% and 65% respectively, compared to the non-amended control (Table 6). This might imply that the test biofertilizer had a mitigating effect on limiting Cd uptake and accumulation in rice. Due to the higher pH and OM content of the biofertilizer BF (Table 2 & 4), both of which were responsible for restricting Cd in soils by facilitating the generation of stable metalo-organo complexes which are more immobile at elevated pH levels (Khan et al. 2017). Cadmium is able to form Cd hydroxide at high soil pH (>7), resulting in the promotion of Cd adsorption to soils. Similarly, OM showed an effect on lowering soil Cd bioavailability and bioaccumulation in rice due to enhanced Cd adsorption and the formation of stable complexes with Cd (Kashem & Singh 2001). Indeed, rice has been regarded as a Cd-sensitive plant and an accumulator of Cd, often containing >0.1 mg Cd kg⁻¹ dry biomass (Grant et al. 2008). Indeed, the nutrients in the biofertilizer itself might also promote native microbial

activity in the amended soils, leading to stimulated nutrient cycling, hormone production, plant symbioses and ultimately enhanced plant tolerance to stress (Farrell et al. 2009; Odlare et al. 2011).

Effect of biofertilizer on photosynthetic pigments in response to Cd stress

One of the deleterious effects due to metal stress is the remarkable destruction of photosynthetic apparatus. The reduction of chlorophyll biosynthesis and its content was observed in various plant species in response to Cd stress (Ahmad et al. 2016; Hawrylak-Nowak et al. 2015). Our study revealed a remarkable decrease in photosynthetic pigment content in terms of the Chl (a+b) and carotenoid content in rice seedling leaves under Cd stress. These losses of pigments due to Cd toxicity might be a result of suppression of relevant enzymes, leading to impaired pigment biosynthesis. In addition, peroxidative breakdown of photosynthetic pigments and the chloroplast membrane lipid may occur in response to abiotic stress due to excessive ROS (Duman et al. 2010). However, these photosynthetic pigment losses could be suppressed when applying the biofertilizer (Table 7), in consistence with previous report (Zhu et al. 2020). Alleviating Chl and carotenoid content might be linked with an increase in Cd sequestration or the pigment biosynthesis and/or decrease in the breakdown of pigment complexes (Sharma et al. 2012).

The effect of biofertilizer on proline content and enzymatic antioxidants upon Cd stress

Upon Cd stress, plants exhibit a range of secondary stress symptoms, including osmotic changes (Wang et al. 2008). Under stress, the plants utilize crucial strategies by adjusting osmolytes to alleviate the water imbalance. For instance, biosynthesis and accumulation of proline, glycine betaine, and trehalose leads to osmotic adjustment of Na^+ stress inside cells to equilibrate water balance (Pandey & Gupta 2015). Our study showed an increased proline content in rice tissues under Cd stress, but this accumulation was suppressed by the test biofertilizer (Table 8). The exogenous application of biofertilizer could mitigate the water imbalance in Cd-stressed rice plants by modulating the biosynthesis of proline.

Recent findings have indicated that APX and SOD are regarded as prominent enzymatic antioxidant in plant's defense system for scavenging toxic $\text{O}_2^{\cdot-}$ radicals and converting them to H_2O_2 (Bhuyan et al. 2020). The dramatical change in APX and SOD activity toward oxidative stress might be due to increased $\text{O}_2^{\cdot-}$ content, which was endured by the test biofertilizer (Table 8). Our findings are also in line with other previous study that reported remarkable changes in activities of APX and SOD as well as other enzymatic and non-enzymatic antioxidants in plant responses to Cd stress (Hawrylak-Nowak et al. 2015). Similarly, recent research has documented an increase in the activities of potent antioxidant enzymes APX and SOD in concentration-related manner (1.0 mM and 2.0 mM CdCl_2 respectively), as compared to the control rice seedlings (*Oryza sativa* L. cv. BRRI dhan54) (Bhuyan et al. 2020).

Taken together, the biofertilizer containing indigenous Cd-resistant bacterial consortium therefore appears to enhance plant growth and tolerance to Cd stress. To our investigation, the soil supplementation of the test biofertilizer BF at 3% applied rate in Thai rice cultivar (PSL2) could mitigate Cd phytotoxicity by modulating antioxidative defense system, water and nutrient homeostasis, and restricting soil Cd

bioavailability. These responses most likely reflect multi-modalities of the biofertilizer harboring such microbial detoxifiers and organic residues, where it helps adjust the antioxidative defense system and water relation together with improvement of soil biophysicochemical traits in coping with Cd toxicity in the biofertilizer-amended plant. Furthermore, the test biofertilizer may offer development of an eco-friendly sustainable strategy for improving plant fitness and soil quality rather than those using foreign strains, with practicability and simplicity.

Conclusion

Amending indigenous Cd-resistant microbe derived-biofertilizer was effective in mitigating the Cd phytotoxicity by modifying the soil biophysicochemical traits to restrict the Cd bioavailability and enhancing plant tolerance toward environmental stress. The promoting effect of biofertilizer could be due to a rise in soil pH and enrichment of beneficial detoxifiers such as *Bacteroidetes*, *Firmicutes* and *Proteobacteria* in the biofertilizer, which stabilized soil Cd and limited its bioavailability, in addition to triggered stress-responsive modulators such as antioxidative enzymes and proline osmolyte. Our results indicated that the test biofertilizer as combined bioagents with fertilizer could not only nourish soil fertility through increasing the OM contents but also be served as a cost-effective amendment, especially at an applied rate to immobilize Cd in the polluted soil. These findings have introduced a promising mean for sustainable development of strategies in bioremediation of Cd-contaminated soils and plant growth improvement. Further study should be designated in the field setting to determine the efficiency of other crop residues and animal manure in the heavy metal decontamination in soils to reduce health risk of exposure to excessive toxic metals via the food chain resulting from anthropogenic environments.

Declarations

Authors' contributions

The funding acquisition was prepared by Preeyaporn Koedrith and Seriwat Saminpanya. The experimental conceptualization and design were conducted by Preeyaporn Koedrith. Methodology, material preparation, data collection and analysis were performed by Ladda Seang-On and Preeyaporn Koedrith. The first draft of the manuscript was written by Preeyaporn Koedrith with suggestion by Weeradej Meeinkuirt. All authors read and approved the final manuscript.

Funding information

This work was supported by a grant from Mahidol University, Thailand under the program titled, "Stabilization and bioremediation of cadmium- and zinc-contaminated soil for sustainable rice cultivation" from the National Research Council of Thailand.

Data availability

All data generated or analyzed during this study are included in this published article and information files.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Completing interests The authors declare no completing interests.

References

1. Abdelhafez AA, Li J, Abbas MH (2014) Feasibility of biochar manufactured from organic wastes on the stabilization of heavy metals in a metal smelter contaminated soil. *Chemosphere* 117: 66-71.
2. Ahemad M, Khan MS (2012a) Ecological assessment of biotoxicity of pesticides towards plant growth promoting activities of pea (*Pisum sativum*)-specific rhizobium SP. STRAINMRP1. *Emir J Food Agric* 334-343. 334-343.
3. Ahemad M, Khan MS (2012b) Effect of fungicides on plant growth promoting activities of phosphate solubilizing *Pseudomonas putida* isolated from mustard (*Brassica campestris*) rhizosphere. *Chemosphere* 86: 945-950.
4. Ahemad M, Khan MS (2012c) Evaluation of plant-growth-promoting activities of rhizobacterium *Pseudomonas putida* under herbicide stress. *Annals of microbiology* 62: 1531-1540.
5. Ahemad M, Khan MS (2012d) Alleviation of fungicide-induced phytotoxicity in greengram [*Vigna radiata* (L.) Wilczek] using fungicide-tolerant and plant growth promoting *Pseudomonas* strain. *Saudi J Biol Sci* 19: 451-459.
6. Ahmad P, Abd Allah E, Hashem A, Sarwat M, Gucel S (2016) Exogenous application of selenium mitigates cadmium toxicity in *Brassica juncea* L.(Czern & Cross) by up-regulating antioxidative system and secondary metabolites. *J Plant Growth Regul* 35: 936-950.
7. Asada K (1987) Production and scavenging of active oxygen in photosynthesis. *Photoinhibition* 227-287. 227-287.
8. Bates LS, Waldren RP, Teare I (1973) Rapid determination of free proline for water-stress studies. *Plant and soil* 39: 205-207.
9. Beyer Jr WF, Fridovich I (1987) Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal Biochem* 161: 559-566.
10. Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microb Cell Fact* 13: 1-10.
11. Bhuyan M, Parvin K, Mohsin SM, Mahmud JA, Hasanuzzaman M, Fujita M (2020) Modulation of cadmium tolerance in rice: Insight into vanillic acid-induced upregulation of antioxidant defense and glyoxalase systems. *Plants* 9: 188.

12. Bray RH, Kurtz LT (1945) Determination of total, organic, and available forms of phosphorus in soils. *Soil science* 59: 39-46.
13. Burnison BK (1980) Modified dimethyl sulfoxide (DMSO) extraction for chlorophyll analysis of phytoplankton.
14. *Can J Fish Aquat Sci* 37: 729-733.
15. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature methods* 7: 335-336.
16. Catherine S, Christophe S, Louis MJ (2006) Response of *Thlaspi caerulescens* to nitrogen, phosphorus and sulfur fertilisation. *Int J Phytoremediation* 8: 149-161.
17. Cazorla F, Romero D, Pérez-García A, Lugtenberg B, Vicente Ad, Bloemberg G (2007) Isolation and characterization of antagonistic *Bacillus subtilis* strains from the avocado rhizoplane displaying biocontrol activity. *J Appl Microbiol* 103: 1950-1959.
18. Chen L, Yang X, Raza W, Luo J, Zhang F, Shen Q (2011) Solid-state fermentation of agro-industrial wastes to produce bioorganic fertilizer for the biocontrol of *Fusarium* wilt of cucumber in continuously cropped soil. *Bioresource Technology* 102: 3900-3910.
19. Deng R, Huang D, Zeng G, Wan J, Xue W, Wen X, Liu X, Chen S, Li J, Liu C (2019) Decontamination of lead and tetracycline from aqueous solution by a promising carbonaceous nanocomposite: Interaction and mechanisms insight. *Bioresource technology* 283: 277-285.
20. Deng R, Huang D, Xue W, Lei L, Zhou C, Chen S, Wen X, Liu X (2020) How does the microenvironment change during the stabilization of cadmium in exogenous remediation sediment? *J Hazard Mater* 398: 122836.
21. Din BU, Rafique M, Javed MT, Kamran MA, Mehmood S, Khan M, Sultan T, Munis MFH, Chaudhary HJ (2020) Assisted phytoremediation of chromium spiked soils by *Sesbania sesban* in association with *Bacillus xiamenensis* PM14: A biochemical analysis. *Plant physiology and biochemistry* 146: 249-258.
22. Duman F, Ozturk F, Aydin Z (2010) Biological responses of duckweed (*Lemna minor* L.) exposed to the inorganic arsenic species As (III) and As (V): effects of concentration and duration of exposure. *Ecotoxicology* 19: 983-993.
23. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27: 2194-2200.
24. Farrell M, Griffith GW, Hobbs PJ, Perkins WT, Jones DL (2009) Microbial diversity and activity are increased by compost amendment of metal-contaminated soil. *FEMS Microbiol Ecol* 71: 94-105.
25. Gajdos É, Lévai L, Veres S, Kovács B (2012) Effects of biofertilizers on maize and sunflower seedlings under cadmium stress. *Commun Soil Sci Plant Anal* 43: 272-279.
26. Gill RA, Zhang N, Ali B, Farooq MA, Xu J, Gill MB, Mao B, Zhou W (2016) Role of exogenous salicylic acid in regulating physio-morphic and molecular changes under chromium toxicity in black-and yellow-seeded *Brassica napus* L. *Environ Sci Pollut Res* 23: 20483-20496.

27. Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. *Scientifica* 2012
28. Govindasamy V, Senthilkumar M, Bose P, Kumar LV, Ramadoss D, Annapurna K (2011) ACC deaminase containing PGPR for potential exploitation in agriculture, *Bacteria in Agrobiological Plant Nutrient Management*. Springer, pp. 183-208
29. Grant CA, Clarke JM, Duguid S, Chaney R (2008) Selection and breeding of plant cultivars to minimize cadmium accumulation. *Sci Total Environ* 390: 301-310.
30. Hawrylak-Nowak B, Dresler S, Matraszek R (2015) Exogenous malic and acetic acids reduce cadmium phytotoxicity and enhance cadmium accumulation in roots of sunflower plants. *Plant Physiol Biochem* 94: 225-234.
31. Herlemann DP, Labrenz M, Jürgens K, Bertilsson S, Waniek JJ, Andersson AF (2011) Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *The ISME journal* 5: 1571-1579.
32. Jahanian A, Chaichi M, Rezaei K, Rezayazdi K, Khavazi K (2012) The effect of plant growth promoting rhizobacteria (PGPR) on germination and primary growth of artichoke (*Cynara scolymus*). *Int Agric Crop Sci (IJACS)* 4: 923-929.
33. Javed MT, Tanwir K, Akram MS, Shahid M, Niazi NK, Lindberg S (2019): Phytoremediation of cadmium-polluted water/sediment by aquatic macrophytes: role of plant-induced pH changes, cadmium toxicity and tolerance in plants. Elsevier, pp. 495-529
34. Jha PN, Kumar A (2007) Endophytic colonization of *Typha australis* by a plant growth-promoting bacterium *Klebsiella oxytoca* strain GR-3. *J Appl Microbiol* 103: 1311-1320.
35. Kashem M, Singh B (2001) Metal availability in contaminated soils: I. Effects of flooding and organic matter on changes in Eh, pH and solubility of Cd, Ni and Zn. *Nutr Cycl Agroecosyst* 61: 247-255.
36. Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *J Trace Elem Med Biol* 18: 355-364.
37. Khan MA, Khan S, Khan A, Alam M (2017) Soil contamination with cadmium, consequences and remediation using organic amendments. *J Total Environ* 601: 1591-1605.
38. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265-275.
39. Lu K, Yang X, Shen J, Robinson B, Huang H, Liu D, Bolan N, Pei J, Wang H (2014) Effect of bamboo and rice straw biochars on the bioavailability of Cd, Cu, Pb and Zn to *Sedum plumbizincicola*. *Agric Ecosyst Environ* 191: 124-132.
40. Lux A, Martinka M, Vaculík M, White PJ (2011) Root responses to cadmium in the rhizosphere: a review. *J Exp Bot* 62: 21-37.
41. Madhaiyan M, Poonguzhali S, 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.

42. Moreira H, Marques AP, Franco AR, Rangel AO, Castro PM (2014) Phytomanagement of Cd-contaminated soils using maize (*Zea mays* L.) assisted by plant growth-promoting rhizobacteria. *Environ Sci Pollut Res* 21: 9742-9753.
43. Nejad ZD, Jung MC, Kim K-H (2018) Remediation of soils contaminated with heavy metals with an emphasis on immobilization technology. *Environ Geochem Health* 40: 927-953.
44. Nelson DW, Sommers LE (1996) Total carbon, organic carbon, and organic matter. *Methods of soil analysis: Part 3 Chemical methods* 5: 961-1010.
45. Odlare M, Arthurson V, Pell M, Svensson K, Nehrenheim E, Abubaker J (2011) Land application of organic waste—effects on the soil ecosystem. *Appl Energy* 88: 2210-2218.
46. Oorts K, Ghesquiere U, Smolders E (2007) Leaching and aging decrease nickel toxicity to soil microbial processes in soils freshly spiked with nickel chloride. *Environ Toxicol Chem: Int J* 26: 1130-1138.
47. Pandey C, Gupta M (2015) Selenium and auxin mitigates arsenic stress in rice (*Oryza sativa* L.) by combining the role of stress indicators, modulators and genotoxicity assay. *J hazard Mater* 287: 384-391.
48. Rafique M, Ortas I, Rizwan M, Sultan T, Chaudhary HJ, Işık M, Aydin O (2019) Effects of *Rhizophagus clarus* and biochar on growth, photosynthesis, nutrients, and cadmium (Cd) concentration of maize (*Zea mays*) grown in Cd-spiked soil. *Environ Sci Pollut Res* 26: 20689-20700.
49. Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends in biotechnology* 28: 142-149.
50. Rizwan M, Ali S, Hussain A, Ali Q, Shakoor MB, Zia-ur-Rehman M, Farid M, Asma M (2017) Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (*Triticum aestivum* L.) and health risk assessment. *Chemosphere* 187: 35-42.
51. Rodrigues EP, Rodrigues LdS, de Oliveira ALM, Baldani VLD, dos Santos Teixeira KR, Urquiaga S, Reis VM (2008) *Azospirillum amazonense* inoculation: effects on growth, yield and N₂ fixation of rice (*Oryza sativa* L.). *Plant and Soil* 302: 249-261.
52. Sangthong C, Setkit K, Prapagdee B (2016) Improvement of cadmium phytoremediation after soil inoculation with a cadmium-resistant *Micrococcus* sp. *Environ Sci Pollut Res* 23: 756-764.
53. Saravanakumar D, Vijayakumar C, Kumar N, Samiyappan R (2007) PGPR-induced defense responses in the tea plant against blister blight disease. *Crop protection* 26: 556-565.
54. Sarwar N, Ishaq W, Farid G, Shaheen MR, Imran M, Geng M, Hussain S (2015) Zinc–cadmium interactions: Impact on wheat physiology and mineral acquisition. *Ecotoxicol Environ Saf* 122: 528-536.
55. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 75: 7537-7541.

56. Seang-On L, Meeinkuirt W, Saengwilai P, Saminpanya S, Koedrith K (2019) Alleviation of cadmium stress in Thai rice cultivar (PSL2) by inoculation of indigenous cadmium-resistant microbial consortia. *Appl Ecol Environ Res* 17: 14679-14697.
57. Shah AA, Ahmed S, Ali A, Yasin NA (2020) 2-Hydroxymelatonin mitigates cadmium stress in *Cucumis sativus* seedlings: Modulation of antioxidant enzymes and polyamines. *Chemosphere* 243: 125308.
58. Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J Bot* 2012: 217037.
59. Shen Z, Zhong S, Wang Y, Wang B, Mei X, Li R, Ruan Y, Shen Q (2013) Induced soil microbial suppression of banana *Fusarium* wilt disease using compost and biofertilizers to improve yield and quality. *Eur J Soil Biol* 57: 1-8.
60. Shen Z, Ruan Y, Chao X, Zhang J, Li R, Shen Q (2015) Rhizosphere microbial community manipulated by 2 years of consecutive biofertilizer application associated with banana *Fusarium* wilt disease suppression. *Biol Fertil Soils* 51: 553-562.
61. Simmons RW, Pongsakul P, Chaney R, Saiyasitpanich D, Klinphoklap S, Nobuntou W (2003) The relative exclusion of zinc and iron from rice grain in relation to rice grain cadmium as compared to soybean: Implications for human health. *Plant and Soil* 257: 163-170.
62. Simmons RW, Pongsakul P, Saiyasitpanich D, 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: implications for public health. *Environ Geochem Health* 27: 501-511.
63. Siripornadulsil S, Siripornadulsil W (2013) Cadmium-tolerant bacteria reduce the uptake of cadmium in rice: potential for microbial bioremediation. *Ecotoxicol Environ Saf* 94: 94-103.
64. Suksabye P, Pimthong A, Dhurakit P, Mekvichitsaeng P, Thiravetyan P (2016) Effect of biochars and microorganisms on cadmium accumulation in rice grains grown in Cd-contaminated soil. *Environ Sci Pollut Res* 23: 962-973.
65. Sun L, Niu Z, Sun T (2007) Effects of amendments of N, P, Fe on phytoextraction of Cd, Pb, Cu, and Zn in soil of Zhangshi by mustard, cabbage, and sugar beet. *Environ Toxic: Intern J* 22: 565-571.
66. Swaddiwudhipong W, Limpatanachote P, Mahasakpan P, Krintratun S, Punta B, Funkhiew T (2012) Progress in cadmium-related health effects in persons with high environmental exposure in northwestern Thailand: a five-year follow-up. *Environ Res* 112: 194-198.
67. Tank N, Saraf M (2010) Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. *J Plant Interact* 5: 51-58.
68. Tanwir K, Akram MS, Masood S, Chaudhary HJ, Lindberg S, Javed MT (2015) Cadmium-induced rhizospheric pH dynamics modulated nutrient acquisition and physiological attributes of maize (*Zea mays* L.). *Environ Sci Pollut Res* 22: 9193-9203.
69. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy metal toxicity and the environment. *Molecular, clinical and environmental toxicology* 133-164. 133-164.
70. Tian F, Ding Y, Zhu H, Yao L, Du B (2009) Genetic diversity of siderophore-producing bacteria of tobacco rhizosphere. *Braz J Microbiol* 40: 276-284.

71. Wang M, Li S, Chen S, Meng N, Li X, Zheng H, Zhao C, Wang D (2019) Manipulation of the rhizosphere bacterial community by biofertilizers is associated with mitigation of cadmium phytotoxicity. *Sci Total Environ* 649: 413-421.
72. Wang Z, Zhang Y, Huang Z, Huang L (2008) Antioxidative response of metal-accumulator and non-accumulator plants under cadmium stress. *Plant and soil* 310: 137-149.
73. Wani PA, Khan MS (2010) *Bacillus* species enhance growth parameters of chickpea (*Cicer arietinum* L.) in chromium stressed soils. *Food Chem Toxicol* 48: 3262-3267.
74. Younis U, Qayyum MF, Shah MHR, Danish S, Shahzad AN, Malik SA, Mahmood S (2015) Growth, survival, and heavy metal (Cd and Ni) uptake of spinach (*Spinacia oleracea*) and fenugreek (*Trigonella corniculata*) in a biochar-amended sewage-irrigated contaminated soil. *J Plant Nutr Soil Sci* 178: 209-217.
75. Zhu Y, Wang H, Lv X, Zhang Y, Wang W (2020) Effects of biochar and biofertilizer on cadmium-contaminated cotton growth and the antioxidative defense system. *Sci Rep* 10: 20112.

Tables

Table 1 Experimental treatments and their nomenclature used in this study.

Symbol	Treatments
CK	Non-amended plants under normal condition
CD	Non-amended plants under Cd stress
OF.D	Plants amended with organic fertilizer (OF) under Cd stress
BC.D	Plants inoculated with indigenous Cd-resistant bacterial consortium (BC) under Cd stress
BF.D	Plants amended with biofertilizer (BF) containing indigenous Cd-resistant bacterial consortium under Cd stress

Table 2 Physicochemical properties and bacterial composition in phyla of biofertilizer

Biofertilizer		BF	OF
Total viable count (CFU g ⁻¹)		7.8x10 ⁸	-
Bacterial composition in phyla (%)	Acidobacteria	1.46	-
	Bacteriodetes	25.89	-
	Firmicutes	55.45	-
	Gemmatimonadetes	0.12	-
	Proteobacteria	16.69	-
	Planctomycetes	0.27	-
	Others	0.12	-
pH		8.07	7.14
OM (mg kg ⁻¹)		4035	3046
N (mg kg ⁻¹)		618	814
P (mg kg ⁻¹)		922	611
K (mg kg ⁻¹)		1205	761
Ca (mg kg ⁻¹)		84	107
Mg (mg kg ⁻¹)		82	98
S (mg kg ⁻¹)		28	22
Fe (mg kg ⁻¹)		39	35
Mn (mg kg ⁻¹)		2.3	5.13
Zn (mg kg ⁻¹)		3.2	4.8
Cd (mg kg ⁻¹)		ND	ND

Note: pH (1:5 soil/water); CEC cation exchange capacity; OM organic matter; Total N total nitrogen; Ext. P extractable phosphorus; Ext. K extractable potassium; Ca Calcium; Mg Magnesium; S Sulfur; Fe Iron; Mn Manganese; Zn Zinc; Cd Cadmium; ND not detectable; BF biofertilizer containing indigenous Cd-resistant bacterial consortium; OF organic fertilizer as amendment control.

Table 3 Summary of 16S rRNA gene Illumina MiSeq sequencing data and diversity estimates for each sample

Sample	Process	Reads	OTUs	Coverage	Chao1	Shannon
BC#1	Enrichment	60999± 8307	3042± 198	0.998	1514.74	4.60
BC#2	Enrichment	61629± 5829	3021± 314	0.998	1670.77	4.54
BC#3	Enrichment	61347± 6018	3092± 268	0.997	1497.53	4.76
BF#1	Biofertilization	61034± 8109	3008± 229	0.996	5451.82**	10.72**
BF#2	Biofertilization	62182± 6102	3034± 312	0.997	5515.27**	10.83**
BF#3	Biofertilization	63179± 7019	3063± 251	0.998	5672.43**	10.92**

Note: **Indicates respective significant difference at p -value ≤ 0.05 , by comparing the selected parameters (Chao1

richness or Shannon diversity estimator) of the bacterial enriched consortia to that of the corresponding

biofertilizers. OTUs operational taxonomic units; BC Cd-resistant bacterial consortia after Cd-supplemented culture

enrichment of the naturally polluted topsoil samples; BF biofertilizers after semi-solid state fermentation process of

the corresponding enriched consortium.

Table 4-1 Effect of biofertilizer containing indigenous Cd-resistant bacterial consortium on soil physicochemical properties in potting system after plantation under Cd stress

Treatment	pH	EC (dS m ⁻¹)	CEC (cmol kg ⁻¹)	OM (%)	Total N (mg kg ⁻¹)	Ext. P (mg kg ⁻¹)	Ext. K (mg kg ⁻¹)
Before planting							
CD	6.65	0.27 ^a	10.4 ^a	1.09 ^a	2118 ^a	8.7 ^a	164.3 ^a
After planting							
CD	6.47	0.12 ^b	9.5 ^a	1.18 ^a	2202 ^a	9.4 ^a	153.4 ^a
OF.D	6.93	0.54 ^c	15.8 ^b	2.47 ^b	6229 ^b	19.4 ^b	212.4 ^a
BC.D	6.97	0.78 ^c	16.6 ^b	2.38 ^b	6083 ^b	20.9 ^b	206.8 ^a
BF.D	7.83	1.68 ^d	19.8 ^b	2.93 ^b	7645 ^b	25.8 ^b	298.5 ^b

Note: All the values are the mean of three replicates $\pm$ standard error of means. Different lowercase letters indicate statistically significant difference between treatments ($p \leq 0.05$). Details of treatments as given in Table 1. BC Cd-resistant bacterial consortium after culture enrichment; BF Biofertilizer containing indigenous Cd-resistant bacterial consortium; OF organic fertilizer as amendment control; pH (1:5 soil/water); EC electrical conductivity; CEC cation exchange capacity; OM organic matter; Total N total nitrogen; Ext. P extractable phosphorus; Ext. K extractable potassium.

Table 4-2 Effect of biofertilizer containing indigenous Cd-resistant bacterial consortium on soil Cd and Zn content in potting system after plantation under Cd stress

Treatment	pH	Total Cd (mg kg ⁻¹)	Ext. Cd (mg kg ⁻¹)	Total Zn (mg kg ⁻¹)	Ext. Zn (mg kg ⁻¹)
Before planting					
CD	6.65	69.1 $\pm$ 10.3 ^a	8.91 $\pm$ 0.22 ^a	729.5 $\pm$ 62.3 ^a	117.8 $\pm$ 18.6 ^a
After planting					
CD	6.47	68.4 $\pm$ 11.2 ^a	8.84 $\pm$ 0.19 ^a	718.8 $\pm$ 69.9 ^a	108.7 $\pm$ 21.6 ^a
OF.D	6.93	36.4 $\pm$ 10.5 ^b	4.89 $\pm$ 0.21 ^b	464.2 $\pm$ 50.2 ^b	68.5 $\pm$ 13.5 ^b
BC.D	6.97	30.4 $\pm$ 10.7 ^b	4.17 $\pm$ 0.23 ^b	338.8 $\pm$ 45.5 ^b	53.7 $\pm$ 11.4 ^b
BF.D	7.83	22.4 $\pm$ 8.6 ^b	4.03 $\pm$ 0.16 ^b	302.2 $\pm$ 53.3 ^b	45.8 $\pm$ 12.6 ^b

Note: All the values are the mean of three replicates $\pm$ standard error of means. Different lowercase letters indicate statistically significant difference between treatments ($p \leq 0.05$). Details of treatments as given in Table 1. BC Cd-resistant bacterial consortium after culture enrichment; BF Biofertilizer containing indigenous Cd-resistant bacterial consortium; OF organic fertilizer as amendment control; Total Cd total cadmium; Ext. Cd extractable cadmium; Total Zn total zinc; Ext. Zn extractable zinc.

Table 5 Effect of biofertilizer containing indigenous Cd-resistant microbial consortia on the biomass and length of shoot and roots of PSL2 Thai rice cultivar grown in Cd-enriched soils in potting system over 4 months

Treatment	Shoot DW (mg plant ⁻¹)	Root DW (mg plant ⁻¹)	Shoot length (cm in average)	Root length (cm in average)
CK	76.4 $\pm$ 4.8 ^a	149.3 $\pm$ 1.8 ^a	45.2 $\pm$ 7.0 ^a	24.6 $\pm$ 4.8 ^a
CD	35.1 $\pm$ 2.8 ^b	78.2 $\pm$ 3.8 ^b	29.3 $\pm$ 5.8 ^b	49.1 $\pm$ 8.4 ^b
OF.D	58.7 $\pm$ 1.7 ^a	127.4 $\pm$ 5.2 ^a	37.6 $\pm$ 5.5 ^a	45.1 $\pm$ 5.3 ^b
BC.D	64.5 $\pm$ 3.5 ^a	138.3 $\pm$ 7.1 ^a	39.2 $\pm$ 5.2 ^a	40.9 $\pm$ 6.1 ^b
BF.D	75.3 $\pm$ 1.4 ^a	165.7 $\pm$ 8.2 ^a	47.2 $\pm$ 8.2 ^a	36.5 $\pm$ 7.3 ^b

Note: All the values are the mean of three replicates $\pm$ standard error of means. Different lowercase letters indicate statistically significant difference between treatments ($p \leq 0.05$). Details of treatments as given in Table 1. BC Cd-resistant bacterial consortium after culture enrichment; BF Biofertilizer containing indigenous Cd-resistant bacterial consortium; OF organic fertilizer as amendment control; DW dry weight;

Table 6 Effect of biofertilizer containing indigenous Cd-resistant microbial consortia on the mineral nutrition (shoot nitrogen and phosphorus content) and tissue Cd content of PSL2 Thai rice cultivar grown in Cd-enriched soils in potting system

Treatment	Shoot N content (mg plant ⁻¹)	Shoot P content (mg plant ⁻¹)	Shoot Cd content (mg kg ⁻¹)	Root Cd content (mg kg ⁻¹)
CK	9.4 ± 0.8 ^a	4.3 ± 0.6 ^a	ND	ND
CD	3.2 ± 0.7 ^b	1.9 ± 0.4 ^b	10.9 ± 2.1 ^a	403.2 ± 87.6 ^a
OF.D	18.7 ± 3.5 ^c	8.5 ± 1.1 ^c	5.1 ± 1.6 ^b	232.9 ± 63.5 ^b
BC.D	21.5 ± 1.3 ^c	10.9 ± 1.4 ^c	4.3 ± 1.8 ^b	165.3 ± 81.3 ^b
BF.D	24.9 ± 3.1 ^c	12.9 ± 1.8 ^c	3.4 ± 1.1 ^b	139.4 ± 71.6 ^b

Note: All the values are the mean of three replicates ± standard error of means. Different lowercase letters indicate statistically significant difference between treatments ($p \leq 0.05$). Details of treatments as given in Table 1. BC Cd-resistant bacterial consortium after culture enrichment; BF Biofertilizer containing indigenous Cd-resistant bacterial consortium; OF organic fertilizer as amendment control; N nitrogen; P phosphorus; ND not detectable.

Table 7 Effect of biofertilizer indigenous containing Cd-resistant microbial consortia on the photosynthetic pigments (chlorophyll a and b and carotenoids content) of PSL2 Thai rice cultivar grown in Cd-enriched soils in potting system

Treatment	Chl a (mg g ⁻¹ FW)	Chl b (mg g ⁻¹ FW)	Total Chl (mg g ⁻¹ FW)	Carotenoid (mg g ⁻¹ FW)
CK	1.6 ± 0.3 ^a	1.0 ± 0.1 ^a	2.6 ± 0.3 ^a	1.8 ± 0.5 ^a
CD	0.6 ± 0.2 ^b	0.4 ± 0.1 ^b	1.0 ± 0.2 ^b	0.7 ± 0.2 ^b
OF.D	1.0 ± 0.5 ^a	0.6 ± 0.2 ^a	1.6 ± 0.4 ^a	1.1 ± 0.3 ^a
BC.D	1.2 ± 0.2 ^a	0.8 ± 0.1 ^a	2.0 ± 0.2 ^a	1.3 ± 0.2 ^a
BF.D	1.5 ± 0.8 ^a	0.9 ± 0.2 ^a	2.4 ± 0.7 ^a	1.5 ± 0.3 ^a

Note: All the values are the mean of three replicates ± standard error of means. Different lowercase letters indicate statistically significant difference between treatments ($p \leq 0.05$). Details of treatments as given in Table 1. BC Cd-resistant bacterial consortium after culture enrichment; BF Biofertilizer containing indigenous Cd-resistant bacterial consortium; OF organic fertilizer as amendment control; Chl chlorophyll; Total Chl = Chl (a+b); FW fresh weight.

Table 8 Effect of biofertilizer containing indigenous Cd-resistant microbial consortia on the physiological modulators (proline content and enzymatic antioxidant activities of SOD and APX) of PSL2 Thai rice

seedling grown in Cd-enriched soils in potting system

Treatment	Proline content ($\mu\text{g g}^{-1}$ FW)	Leaf SOD activity (EU mg^{-1} protein)	Root SOD activity (EU mg^{-1} protein)	Leaf APX activity (EU mg^{-1} protein)	Root APX activity (EU mg^{-1} protein)
CK	0.7 ± 0.1^a	24.1 ± 3.4^a	26.3 ± 1.8^a	62.9 ± 8.4^a	84.3 ± 3.8^a
CD	2.6 ± 0.2^b	39.2 ± 5.8^b	48.4 ± 3.8^b	105.3 ± 7.6^b	143.7 ± 12.1^b
OF.D	2.2 ± 0.1^b	60.6 ± 6.1^b	62.4 ± 5.2^b	156.5 ± 8.6^b	183.5 ± 11.6^b
BC.D	1.9 ± 0.2^b	98.4 ± 7.9^c	97.4 ± 7.1^c	232.6 ± 9.1^c	287.2 ± 11.8^c
BF.D	1.4 ± 0.3^b	106.2 ± 7.3^c	103.7 ± 8.2^c	269.4 ± 17.1^c	302.4 ± 10.1^c

Note: All values are the mean of three replicates $\pm$ standard error of means. Different lowercase letters indicate statistically significant difference between treatments ($p \leq 0.05$). Details of treatments as given in Table 1. BC Cd-resistant bacterial consortium after culture enrichment; BF Biofertilizer containing indigenous Cd-resistant bacterial consortium; OF organic fertilizer as amendment control. FW fresh weight; EU enzymatic unit; SOD superoxide dismutase; APX ascorbate peroxidase.

Figures

Figure 1

Relative abundance levels of dominant bacterial phyla (a) and genera (b) in the enriched Cd-resistant bacterial consortium (BC) (cultivable Cd) and the corresponding biofertilizer (BF) based on 16S rRNA gene Illumina MiSeq sequencing. The dominant phyla in the biofertilizer and the enriched bacterial consortium include *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Gemmatimonadetes*.

Figure 2

Schematic diagram showing mitigating potential of indigenous Cd-resistant soil microbe-derived biofertilizer to Cd toxicity. Application of biofertilizer containing indigenous Cd-resistant soil microorganisms could alleviate Cd stress to PSL2 Thai rice cultivar grown in contaminated soil, due to improved soil biophysicochemical and nutrient availability leading to lower tissue Cd content and restored growth. Especially, soil pH increased after applying biofertilizer could limit soil Cd bioavailability and toxicity to rice roots. Beneficial phyla detected in the biofertilizer *i.e.*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*, could help plant coping with Cd-induced oxidative stress by adjusting antioxidative defense, water relation and photosynthetic systems.