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

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

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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 tissue Cd content (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.

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

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;Simmons et al. 2005). 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^{o}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 N_2 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 oxidization 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, pH7.0) 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 oxidization 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 µmol l^{-1}) and 2.5 µl of target DNA (5 ng μl^{-1}). The PCR cycling conditions consisted of an initial denaturation step at 94ºC (3 min), followed by 25 cycles of 98°C (20 sec), 55ºC (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 $CuSO_4$ reagent and shaken for 10 min. Finally, the Follin 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 dH_2O 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.

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 (Oktay et al. 1995).

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 H_2O_2, 0.5 mM ascorbic acid, and 100 µl of enzyme crude extract.

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% H_2O_2 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 $CaCl_2$+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 and discussion

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.

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

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. This was elucidated by the fact that soil pH and OM are considered important factors for limiting Cd availability (Khan et al. 2017;Wang et al. 2019).

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 & 6). 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 remain effective in suppressing Cd bioaccumulation (Table 2) (Catherine et al. 2006;Sun et al. 2007).

The level of DTPA-extractable metals including Cd, Zn, Fe, Mg, and Mn could be recommended as an indicator of the pool metal availability in soils. All the amendments showed decrease in the levels of DTPA-extractable Cd 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 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 availability or stability of Cd in soils.

297 In this study, the increase in soil chemical properties such as pH, EC, CEC, and OM content were observed when
298 soil was treated with the test biofertilizer (Table 4-1). Mineral nutrition containing basic cations (K, Ca, and Mg) in
299 the biofertilizer could contribute to the increase in soil pH, EC and CEC, while organic residues derived from
300 agricultural wastes were involved in nourishment of soil organic carbon. Increase in soil EC and CEC could also be
301 regarded as key factors in modulating heavy metal exchangeability and bioavailability (Abdelhafez et al. 2014;Lu et
302 al. 2014). Increasing soil pH after applying biofertilizer could increase the negatively charged surfaces as well as
303 alkaline conditions, such as hydroxide and carbonate groups, which might support active substances for surface
304 sorption, precipitation and complexation; thus, reducing heavy metal bioavailability with less possibility to enter the
305 food chain (Younis et al. 2015). Compared to the control, the application of biofertilizer BF remarkably increased soil
306 pH, but the amendment OF had less impact on increasing soil pH (Table 4-1). This could be due to the initial pH of
307 the amendments (Table 2). Although applying the amendment OF had slight effect on soil pH, this amendment still
308 decreased Cd contents in rice tissues compared to the control (Tables 4 & 6). These results indicated that besides pH,
309 the mineral nutrients, e.g., N, P, K, Ca, Mg, S, Fe, and microorganisms in the biofertilizer might play crucial roles in
310 controlling Cd uptake and bioaccumulation (Table 2) (Catherine et al. 2006;Sun et al. 2007). Related studies reported
311 that soil pH, DTPA-extractable Cd, total phosphorus and organic carbon were determined as the most pivotal
312 environmental factors contributing the changes in microbial community composition (Wang et al. 2019). Additionally,
313 soil organic carbon served as the carbon source to bacteria was considered crucial.
314
315 **The effects of biofertilizer on rice growth under Cd stress**
316 Plant growth is a plausible determinant that reflects Cd phytotoxicity in polluted soils. The plants showed less biomass
317 after exposure to Cd as compared to the non-stressed control. Cd mitigating potential of biofertilizer was observed for
318 the rice plants grown in Cd-enriched soil over 4 months. Exposure of rice plants to Cd stress negatively affected the
319 germination (Seang-On et al. 2019) and their growth performance (Table 5).
320 All the amendments increased tendency of biomass and length of rice shoot and root under Cd stress, as
321 compared to the non-amended stressed control CD (Table 5). Upon Cd toxicity, the application of biofertilizer showed
322 maximum increase in shoot and root dry weight by 115% and 112% respectively, as compared to the control (Table
323 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.

These results indicated that the test biofertilizer was effective in promoting growth performance to rice plant under Cd stress. This may be elucidated by the fact that 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

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. Consistently, stress-responsive microorganisms as well as mineral nutrition in the test amendments could restrict Cd bioavailability in the soil and reduce its toxicity to roots; thereby, facilitating plants to uptake more essential nutrients (Nejad et al. 2018).

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). The nutrients in the biofertilizer might also promote indigenous 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).

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, as 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).

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 control.

Among metals, Cd is recognized as highly toxic metal that impacts the growth and physiological processes development of the plant. One of the deleterious effects due to metal stress is the remarkable decrease of photosynthetic pigments. The reduction of chlorophyll biosynthesis and its content was observed in various plant species in response to Cd stress (Ahmad et al. 2016). Our results 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 as well as the lipid of the chloroplast membrane, may occurs in response to abiotic stress due to excessive ROS production (Duman et al. 2010). However, these phenomena on photosynthetic pigment losses could be improved when applying the biofertilizer, in consistence with previous report (Ahmad et al. 2016). 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 (Ahmad et al. 2016;Hawrylak-Nowak et al. 2015).

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

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 (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 increasing activities of SOD and APX in rice shoots and roots. Recent findings have indicated that APX and SOD are regarded as prominent enzymatic antioxidant in plant's defense system for scavenging toxic O_2.- radicals and converting them to H_2O_2 (Bhuyan et al. 2020). Herewith, the dramatical change in APX and SOD activity might be due to increased O_2.- content, which was suppressed by biofertilizer. These results are in consistence with other previous study on plant responses to abiotic stress Cd, as shown by remarkable changes in activities of APX and SOD as well as other enzymatic and non-enzymatic antioxidants (Hawrylak-Nowak et al. 2015). Moreover, recent research has reported 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 seedlings of rice (Oryza sativa L. cv. BRRI dhan54) (Bhuyan et al. 2020).

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 plants 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.

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 balance

changes. 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). Herein, it revealed 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 as the plants could ameliorate the biosynthesis of proline.

Conclusion

As illustrated in Fig. 2, 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. Also, the organic residue additives in the biofertilizer may act as niche for the microorganisms to support their growth and specific activity, leading to nutrient cycling and plant tolerance to stress. 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.

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Completing interest

The authors have neither finance nor conflict of interests.

Author contributions

435 The experimental conceptualization and design were conducted by Preeyaporn Koedrith, Weeradej Meeinkuirt and
436 Seriwat Saminpanya. Material preparation, data collection and analysis were performed by Ladda Seang-On and
437 Preeyaporn Koedrith. The first draft of the manuscript was written by Preeyaporn Koedrith and all authors read and
438 commented the final manuscript.

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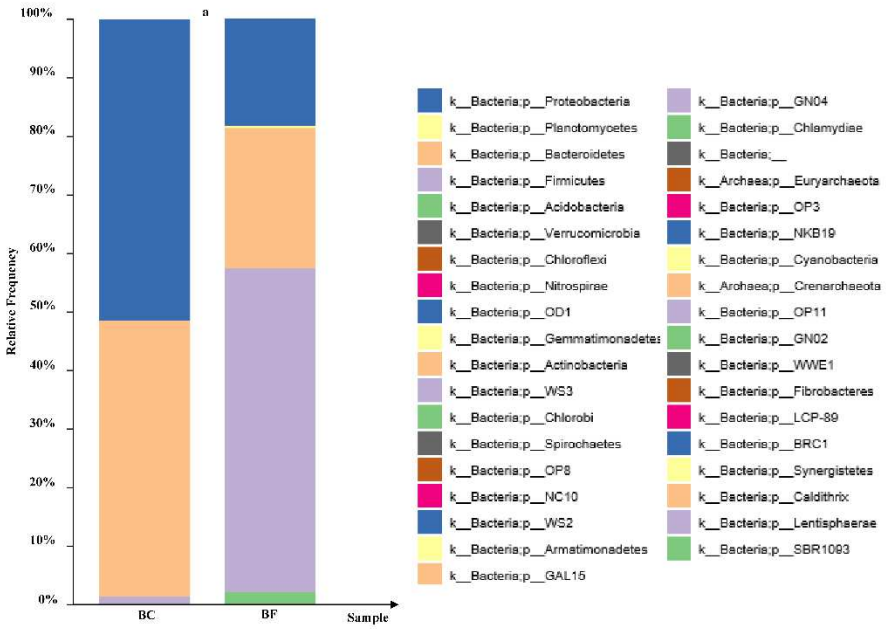

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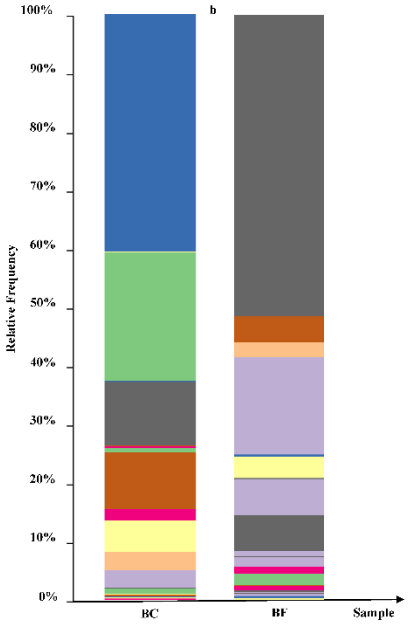

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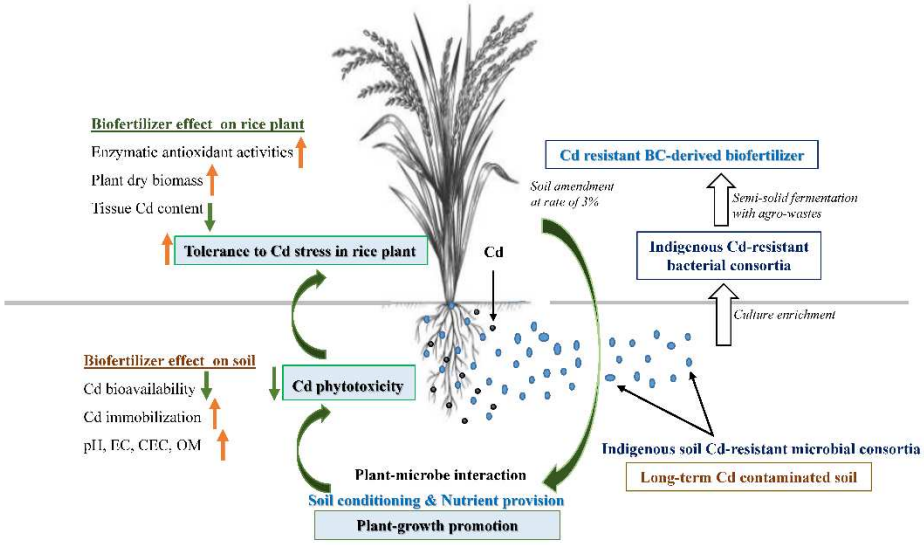
619 **Figure 1** Relative abundance levels of dominant bacterial phyla (a) and genera (b) in the enriched Cd-resistant
620 bacterial consortium (BC) (cultivable Cd) and the corresponding biofertilizer (BF) based on 16S rRNA gene Illumina
621 MiSeq sequencing. The dominant phyla in the biofertilizer and the enriched bacterial consortium include
622 *Proteobacteria, Bacteroidetes, Firmicutes* and *Gemmatimonadetes*.



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624 **Figure 2** Schematic diagram showing mitigating impact of indigenous Cd-resistant soil microbe-derived biofertilizer
625 to Cd toxicity. Application of biofertilizer containing indigenous Cd-resistant soil microorganisms could alleviate Cd
626 stress to PSL2 Thai rice cultivar grown in contaminated soil. Cd phytotoxicity on growing Thai rice cultivar was
627 mitigated, due to soil physicochemical improvement and nutrient availability after applying biofertilizer. Soil pH
628 increased after applying biofertilizer could limit soil Cd bioavailability and toxicity to rice roots. Beneficial phyla
629 detected in the biofertilizer *i.e.*, *Bacteroidetes, Firmicutes* and *Proteobacteria*, could enhance tolerance to Cd stress
630 by adjusting proline content and enzymatic antioxidants.

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637 **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

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654 **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

655 Note: pH (1:5 soil/water); CEC cation exchange capacity; OM organic matter; Total N total nitrogen; Ext. P

656 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 (BC) 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.

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676 **Table 4-1** Effect of biofertilizer containing indigenous Cd-resistant bacterial consortium on soil physicochemical
677 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}

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

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692 **Table 4-2** Effect of biofertilizer containing indigenous Cd-resistant bacterial consortium on soil Cd and Zn content
693 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 ± 10.3^{a}	8.91 ± 0.22^{a}	729.5 ± 62.3^{a}	117.8 ± 18.6^{a}
After planting					
CD	6.47	68.4 ± 11.2^{a}	8.84 ± 0.19^{a}	718.8 ± 69.9^{a}	108.7 ± 21.6^{a}
OF.D	6.93	36.4 ± 10.5^{b}	4.89 ± 0.21^{b}	464.2 ± 50.2^{b}	68.5 ± 13.5^{b}
BC.D	6.97	30.4 ± 10.7^{b}	4.17 ± 0.23^{b}	338.8 ± 45.5^{b}	53.7 ± 11.4^{b}
BF.D	7.83	22.4 ± 8.6^{b}	4.03 ± 0.16^{b}	302.2 ± 53.3^{b}	45.8 ± 12.6^{b}

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

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Table 5 Effect of biofertilizer containing indigenous Cd-resistant microbial consortia on the dry weight and length of shoot and roots of rice cultivar (PSL2) 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 ± 4.8^{a}	149.3 ± 1.8^{a}	45.2 ± 7.0^{a}	24.6 ± 4.8^{a}
CD	35.1 ± 2.8^{b}	78.2 ± 3.8^{b}	29.3 ± 5.8^{b}	49.1 ± 8.4^{b}
OF.D	58.7 ± 1.7^{a}	127.4 ± 5.2^{a}	37.6 ± 5.5^{a}	45.1 ± 5.3^{b}
BC.D	64.5 ± 3.5^{a}	138.3 ± 7.1^{a}	39.2 ± 5.2^{a}	40.9 ± 6.1^{b}
BF.D	75.3 ± 1.4^{a}	165.7 ± 8.2^{a}	47.2 ± 8.2^{a}	36.5 ± 7.3^{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; DW dry weight;

Table 6 Effect of biofertilizer containing indigenous Cd-resistant microbial consortia on the mineral nutrition (nitrogen and phosphorus content in shoot), and tissue Cd content of rice cultivar (PSL2) 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 $\pm$ 1.3^{c}	10.9 ± 1.4^{c}	4.3 ± 1.8^{b}	165.3 $\pm$ 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 photysynthetic pigments (chlorophyll a and b and carotenoids content) of rice cultivar (PSL2) 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.

762 **Table 8** Effect of biofertilizer containing indigenous Cd-resistant microbial consortia on the physiological
763 modulators (proline content and enzymatic antioxidant activities of SOD and APX) of rice seedling cultivar (PSL2)
764 grown in Cd-enriched soils in potting system

Treatment	Proline content (μg 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}

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

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