

Mitigation of Cadmium Phytotoxicity in Thai Rice (*Oryza Sativa L.*) Cultivar by Inoculation of Indigenous Cadmium-Resistant Microbial Consortia

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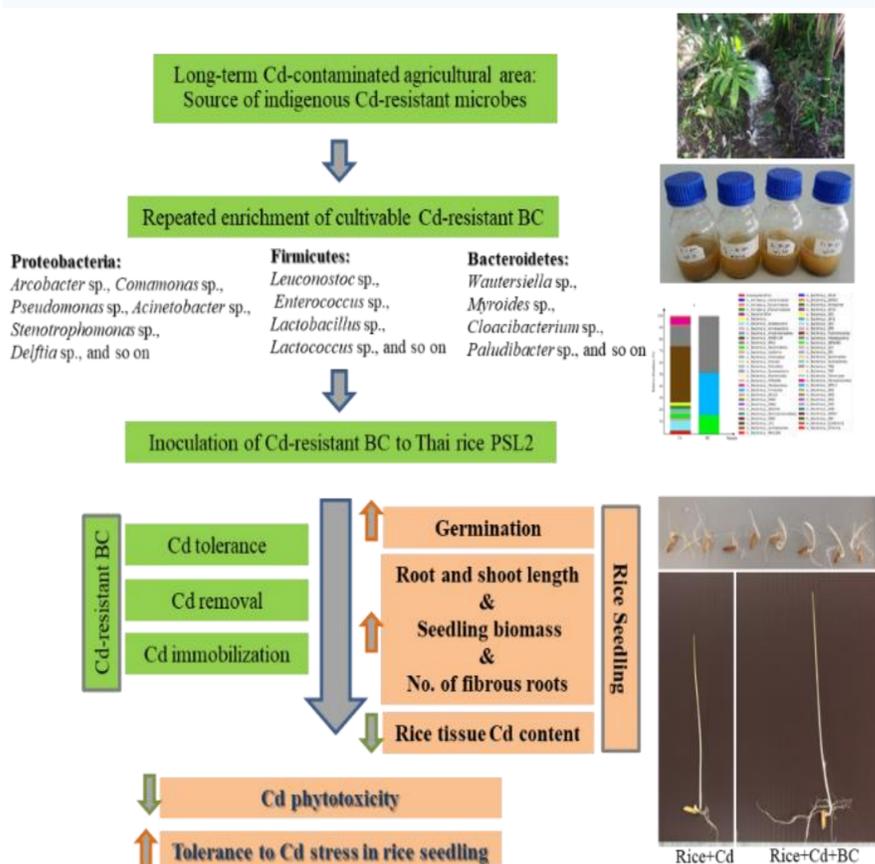
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Background and Aim

- Significantly high levels of Cd (ranging from 3.4-284.0 mg kg⁻¹ <European Community limit of 3 mg kg⁻¹>) in agricultural soils, have been detected and reported in the Padaeng zinc mining area of southeast region of Mae Sot District, Tak Province of Thailand.
- To isolate indigenous Cd-resistant microbial consortia from the contaminated agricultural soils by repeated enrichment culture.
- To characterize their Cd-tolerance and Cd-removal capacities, and assessing their effects on the seed germination and seedling growth of Thai rice cultivar (*Oryza sativa L.*) PSL2, as well as tissue Cd content upon high Cd stress.

Method

Enrichment of Cd-resistant bacteria for 50 or 100 ppm cadmium chloride (CdCl₂) was performed prior to the inoculation in order to ameliorate Cd phytotoxicity by promoting rice biomass and growth, and lowering tissue Cd content upon high Cd exposure. Bacterial diversity and composition of the enriched consortia compared to the originally polluted soil consortia were analysed using 16S rRNA gene Illumina MiSeq sequencing. Cd removal capacity of the enriched consortia in batch culture was estimated through the % Cd removal = $(CI - CF) / CI \times 100$ formula. The effect of Cd-resistant bacteria on rice seedlings was then determined by measuring the root/shoot length and number of fibrous roots of seedlings after incubation at 28°C on 7th and 14th days in the dark, and effect of Cd-resistant bacteria on Cd accumulation of rice tissues was determined in triplicate by measuring the plant root/shoot Cd content.



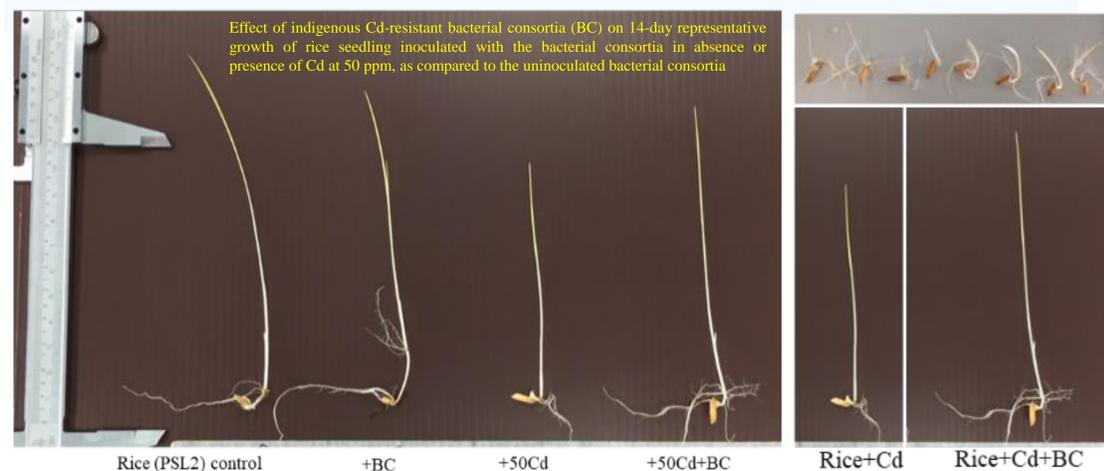
Result and Discussion

- Inoculation with enriched bacterial consortium at 1×10^6 CFU ml⁻¹ led to:
 - Mitigated metal phytotoxicity due to reduced Cd bioconcentration.
 - Promoted biomass production in rice seedlings toward high Cd exposure level, as evidenced by facilitated rice germination and seedling growth even under Cd stress (ca.50% and higher).
 - The consortium had tolerance to Cd (up to 800 ppm).

Table: Effect of indigenous Cd-resistant bacterial consortia (BC) on 14-day rice shoot and root dry biomass and Cd content in solution system containing CdCl₂ at 50 or 100 ppm, with *Bacillus cereus* as control

Treatment	Root dry biomass (mg)	Shoot dry bio-mass (mg)	Root Cd content (mg kg ⁻¹)	Shoot Cd content (mg kg ⁻¹)
Rice (PSL2) Control	14.2 ± 2.0	41.4 ± 4.8	-	-
<i>Bacillus cereus</i>	13.3 ± 1.4	38.2 ± 1.7	-	-
50 ppm CdCl ₂	13.9 ± 1.9 (1.3%)	31.2 ± 3.5 (24.6%)	85.3 ± 1.3	43.2 ± 1.7
50 ppm CdCl ₂ + <i>B. cereus</i>	14.0 ± 2.6	33.0 ± 1.7	90.8 ± 1.4	45.3 ± 1.4
Control	14.2 ± 2.0	41.4 ± 4.8	-	-
Cd-resistant BC	25.4 ± 4.4	44.4 ± 3.2	-	-
50 ppm CdCl ₂	13.9 ± 1.9 (1.3%)	31.2 ± 3.5 (24.6%)	85.3 ± 1.3	43.2 ± 1.7
50 ppm CdCl ₂ + BC	23.9 ± 3.5 ^{##}	35.5 ± 2.7	51.2 ± 1.9 ^{##}	30.5 ± 1.6 ^{##}
100 ppm CdCl ₂	11.9 ± 0.7 (16.1%)	23.0 ± 5.3 (44.5%) ^a	162.1 ± 1.5	72.6 ± 2.0
100 ppm CdCl ₂ + BC	19.8 ± 1.8 ^{##}	34.07 ± 1.6 ^{##}	99.9 ± 2.2 ^{##}	54.0 ± 1.1 ^{##}

(Note: 'a' indicates significant difference at P-value ≤ 0.05, by comparing the selected parameters of Cd-treated group to that of control (untreated group), and ## indicates significant difference at P-value ≤ 0.05, by comparing the selected parameters of bacteria inoculated group to that of uninoculated group in presence of CdCl₂ at indicated concentrations. Numbers in bracket represents percentage of decrease in plant dry biomass of Cd-treated group relative to control. BC indicates Cd-resistant bacterial consortia after Cd-added culture enrichment)



Conclusion

- ❖ Enriched indigenous Cd-resistant microbial consortia (top 3 phyla esp. *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*) exhibited alleviating Cd phytotoxicity and lowering Cd bioaccumulation in the Thai rice cultivar (*Oryza sativa L.*) PSL2, even under high Cd-stress. Owing to their Cd resistance and removal properties, they would further improve crop plant growth and yield for agricultural benefits.

Acknowledgements

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