Assessing endophytic bacteria as a strategy to bioremediate Cd in Cacao crops from Colombia

Daniel Bravo, Sandra Cortés, Sergio Pardo

Laboratory of Soil Microbiology and Calorimetry. Colombian Research Center for Agriculture - Corpoica CO-0571, Mosquera, Colombia (dbravo@corpoica.org.co)

Aim

To assess endophytic cadmium tolerant bacteria (ECdtB) isolated from Cacao crops from Colombia by calorimetric and other techniques in order to immobilize Cd.

Cadmium in Cocoa

Material vegetal

Cadmium is one of the biggest challenges into the quality of Cacao (*Theobroma cacao* L.). One of the strategies to tackle such issue is the use of endophytic cadmium tolerant-bacteria (ECdtB) through metabolizing the bioactive fraction of cadmium, i.e. cadmium oxalate, into geo-stable compounds, i.e. cadmium carbonate (Figure 1).

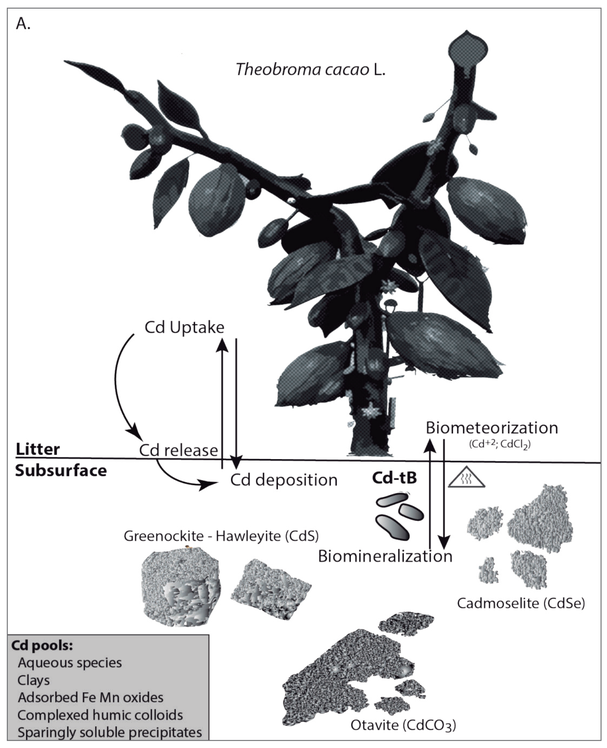

Figure 1. Schematic representation of the cadmium cycle through the cacao.

Isolation and characterization of ECdtB

We have set a pool of 45 E-CdtB isolated from 23 varieties of Cacao represented in 7 genera. The strains *Bacillus* sp. ECdtB1, 2, 3, *Herbaspirillum* sp. ECdtB14 and *Rhizobium* sp. ECdtB5 were selected due the amplification of *cad* and *smt* genes, related with cadmium efflux and chelation in the bacterial cytoplasm (Table 1 and Figure 3), suggesting that its importance in sequestering it can be even greater than expected.

Table 1. Complete profile of selected endophytic cadmium-tolerant bacteria.

		ECdtBEC2	ECdtBEC3	ECdtBEC5
Genes	16S rRNA	*Bacillus* sp.	*Bacillus* sp.	*Rhizobium* sp.
	cadA	+	+	-
	smt	+	+	+
Medium	Mergeay agar $CdCl_2$ 6, 12, mg.l^{-1}	+	+	+
Biolog Gen III Metabolic Profiling	Acetic Acid	-	-	+
	Acetoacetic Acid	-	-	+
	Aztreonam	+	+	+
	Dextrin	-	-	+
	D-Galactose	+	+	-
	D-Gluconic Acid	-	-	+
	D-Glucose-6-PO_4	-	-	+
	D-Serine	-	-	+
	Formic Acid	-	-	+
	Gelatin	-	-	+
	Guanidin- HCl	+	+	+
	L-Arginine	-	-	+
	L-Aspartic Acid	-	-	+
	L-Glutamic Acid	-	-	+
	L-Histidine	-	-	+
	Lincomycin	+	+	-
	Lithium Chloride	-	-	+
	L-Lactic Acid	-	-	+
Biolog Gen III Metabolic Profiling	L-Malic Acid	+	+	+
	L-Serine	-	-	+
	N-Acetyl-D-Galactosamine	+	-	-
	NaCl 1%	+	+	+
	NaCl 4%	-	-	+
	Nalidixic Acid	+	+	-
	Niaproof 4	+	+	-
	pH 6	+	+	+
	Potassium tellurite	-	-	+
	Rifamycin SV	-	-	+
	Sodium Bromate	+	+	+
	Sodium Butyrate	-	-	+
	Sodium lactate 1%	+	+	+
	Tween 40	-	-	+
	α-D-Glucose	+	+	-
IMC data	lambda (λ)	0.44 ± 0.016	0.45 ± 0.021	0.13 ± 0.024
	growth rate (μ h^{-1})	0.27 ± 0.024	0.23 ± 0.019	0.002 ± 0.089
	maximal growth rate (μmax h^{-1})	0.46 ± 0.032	0.57 ± 0.078	0.016 ± 0.095
	time to reach the maximal heat pick (TTP h^{-1})	11.35 ± 0.056	0.51 ± 0.011	1.12 ± 0.044
Cd	Cd immobilization rate (mg l^{-1} h^{-1})	0.0037 ± 0.001	0 ± 0.000	0.008 ± 0.001

Calorimetry & Cd-Immobilization

The thermogram profile represents here (Fig.2), a metabolic fingerprint of endophytic bacteria with Cd-tolerance capacity. Thus, the thermogram did shown that the strain *Bacillus* sp. ECdtBEC2 has the highest metabolic activity with a TTP reached at 11.35 h and an enthalpy change of 2.88 x 10^{-7} kJ mol^{-1} of $CdCl_2$. This strain has been selected for greenhouse experiments.

The strains ECdtBEC2, 3 & 5 were also selected due their higher Cd-immobilization ratios (1.08, 1.07, and 1.9 mg l^{-1} of Cd in 12 days, respectively) in Batch experiments (Fig. 3). ECdtB2 has shown a major activity during Cd-immobilization and minor biomass production (1050 J.s^{-1} and 0.8 OD_{600nm}, respectively), whereas strains ECdtB3 & 5 lower activity and higher biomass production (both 580, 405 J.s^{-1} and 1.4, 1.1 OD_{600nm}, respectively).

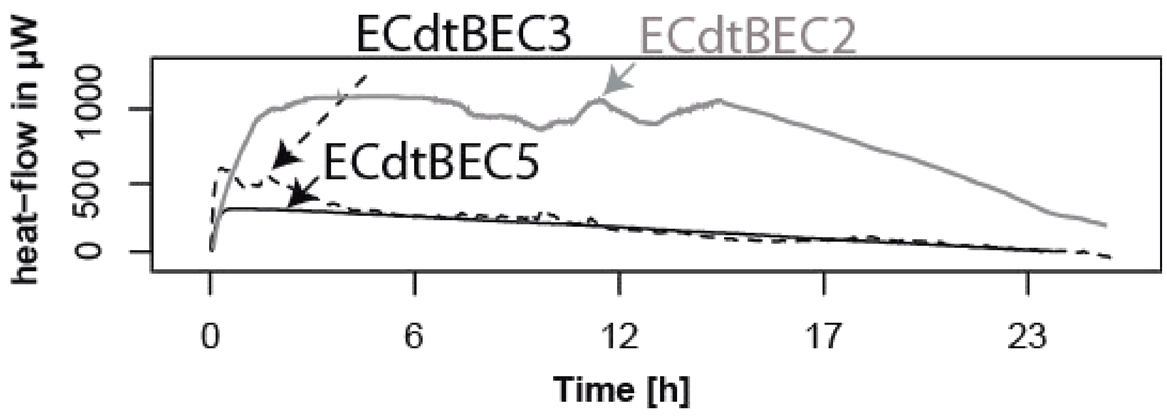

Figure 2. A thermogram of cadmium immobilization activity measured by isothermal microcalorimetry, based in previous work (Bravo et al. 2011) This plot show the maximal metabolic activity in selected ECdtB strains. ECdtBEC2 = *Bacillus* sp. isolated from El Carmen de Chucurí, Santander. ECdtBEC3 and 5 = Bacillus and *Rhizobium* sp.

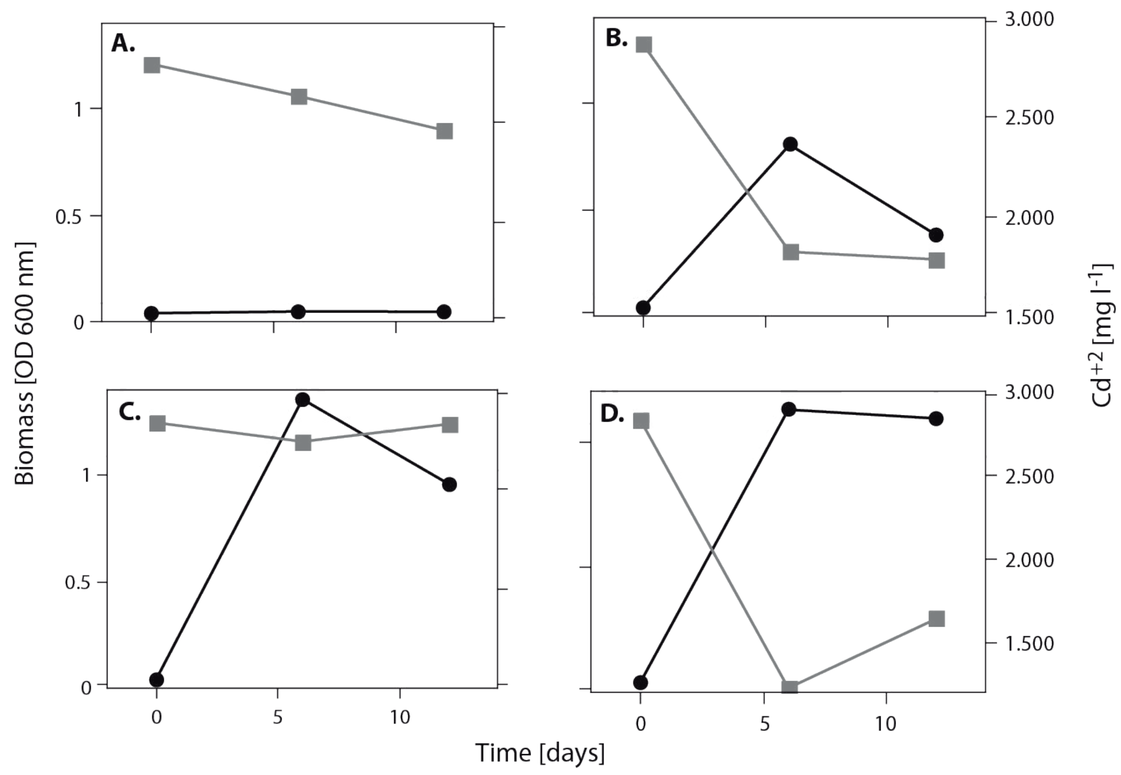

Figure 3. In order to compare the metabolic activity of Cd immobilization observed in Fig. 2, a Batch experiment was carried out, according to previous studies (Bravo et al. 2015). Panel **A.** Negative control, only broth with 6 mg l^{-1} of $CdCl_2$ without bacteria; **B.** *Bacillus* sp. strain ECdtBEC2; **C.** *Bacillus* sp. strain ECdtBEC3 and **D.** *Rhizobium* sp. ECdtBEC5 strain. Black Circles = Biomass. Grey squares = Cd.

Conclusions

This study provides the first key towards cadmium sequestration using endophytic Cd-tolerant microorganisms involved in immobilization processes that might are occurring in Colombian Cocoa agro-systems.

The combining of microcalorimetric and Cd-immobilization assessments allow us to selected ECdtB strains with higher metabolic capacity to be re-inoculated and confirm it endophytism through greenhouse experiments using rootstocks of cacao varieties and measuring its real potentiality to bioremediate Cd.

References

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