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Using Isothermal microcalorimetry to measure cadmium related microbial activity and antagonic activity of fungal populations of cacao-growing farms in Colombia

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In Colombia, cacao is the second largest crop in importance due to its economic and social inclusion to vulnerable populations. However, there are two major concerns of cacao small stakeholders to increase it productivity and enhance the exportations. One is the presence of plantation diseases, mainly caused by fungal pathogens such as *Scopulariopsis* spp which may reduce the production of cacao beans at 40% in worst scenarios. The second major concern is the presence of cadmium in the cacao-growing soils that has become one of the biggest challenges to sustain both production and quality, in the most productive cacao districts of Colombia (Bravo et al. 2021). Therefore, several approaches have converged into control of diseases and mitigation strategies to diagnostic cadmium in soils and prevent its fluxes until its accumulation in the cacao beans. Interestingly, the flux regulation of both pathogen populations and cadmium in cacao soils, is in part mediated, by the microbial diversity related to this system able to deal with the population density of Scopulariopsis and heavy metal, where the activity of cadmium tolerant bacteria (CdtB) (Bravo and Braissant 2021), is from a major interest due to its metabolic pathways to interact with the pathogen Scopulariopsis and Cd (i.e., due to its bioweathering capacity). This dissertation shows to case of study proposing the use of isothermal microcalorimetry (IMC), as a novel approach to measuring the heat released during both antagonic interaction between microbial strains against *Scopulariopsis*, and cadmium exposition, to assess the immobilization potential of CdtB active populations. In the study of antagonic activity, determining the metabolic profile of *Thricohderma asperellum* (Th034 and Th406) and the metabolic interaction between Th034, Th406, and *Bacillus amyloliquefaciens* (Bs006) with *Scopulariopsis* sp. (Sc002) using a combination of isothermal microcalorimetry (IMC), confocal microscopy and BIOLOG test. The strain Bs006 exhibit great growth rate, compared to the fungal strains, however, the Qmax produced by Th406 had the best performance controlling populations of Sc002. The IMC method in combination with Biolog assays, effectively revealed some key phenotypic traits related to substrate assimilation when the interaction occurs within Th406 and Sc002. In the study of cadmium immobilization, the region selected was the northeastern zone of Antioquia district, in Colombia, where 4 cacao farms were studied taking soil samples and comparing the Cd distribution with cadmium tolerant bacteria diversity and function. As positive controls autochthonous CdtB populations from an enriched region in Santander district were used. The calorimetric thermograms shown higher metabolic activities during the time of thermal analysis (80 hours), indicating and increase on cadmium metabolization mediated by both fast and slow-growing populations. The metabolic fluxes and the maximum heat-flow produced were not exceed 500 mW and of 60.8 Joules of heat. Therefore, the biological meaning is that metabolic activity of filamentous fungi was discharged since no heat-production related to that population density was detected. The maximum heat-flow rates were exhibited by the soil samples S01, S05, S16 and S34, where S01 and S05 related to Cd content between 1.11 – 1.40 $mg.kg^{-1}$ on average, samples S16 and S34 belong to farm 3 where the lower Cd content in soil was found (below 1 $mg.kg^{-1}$). Interestingly, the soils samples S01 and S05 were found close to the trunks related to the cacao varieties of ICS95 and EET8 respectively. These varieties have been suggested as hyperaccumulators (Gil et al. 2021) For soil samples S16 and S34, both close to the trunk of the cocoa variety TSH565, allowing to generate a discussion based the accumulation of Cd in tissues for certain specific cocoa varieties according to its biotranslocation/bioaccumulation factors. It is concluded that the IMC method is an excellent method to quantify ecological relationships between antagonic populations in cacao crops, reducing the pathogenicity of aggressive natural fungi populations, and increases our understand about the role of CdtB active populations in cacao-growing soils as an important step to follow up bioremediation strategies where the selection of CdtB populations at specific soil conditions might contribute to reduce the labile fraction of cadmium going into the flux within the plant to a final deposition in cacao beans.

References.

Bravo, D. and Braissant, O. (2021) Cadmium tolerant bacteria: current trends and applications in agriculture. *Lett Appl Micorbiol* **Accepted**.

Bravo, D., Leon-Moreno, C., Martínez, C.A., Varón-Ramírez, V.M., Araujo-Carrillo, G.A., Vargas, R., Quiroga-Mateus, R., Zamora, A. and Rodríguez, E.A.G. (2021) The first national survey of cadmium in cacao farm soil in Colombia. *Agronomy* **11**, 1-18.

Gil, J.P., López-Zuleta, S., Quiroga-Mateus, R.Y., Benavides-Erazo, J., Chaali, N. and Bravo, D. (2021) Cadmium distribution in soils, soil litter and cacao beans: a case study from Colombia. *Int J Sci Environ Technol*, 1-22.