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# CONTROLLING HEAVY METAL ACCUMULATION IN PLANTS

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The present study is aimed at understanding the interactions between plants, microorganisms and heavy metals. We have considered a natural environment, in Northern France, that shows high cadmium, zinc and lead pollution. Plants of the metal hyperaccumulator *Arabidopsis halleri* were collected together with their rhyzospheric soil. Several bacterial strains were then isolated from this soil and identified. Among these, a strain of *Pseudomonas putida* was considered for further analyses. Proteomic analysis was performed on this strain (called *P. putida-Cd001*) grown with and without Cd added to the nutrient medium. The differential patterns of protein expression were visualized by two dimensional electrophoresis technique and proteins were identified by MS analysis. Results showed that many different membrane proteins were up-regulated upon Cd treatment, in particular membrane transporters.

This study is focalized on the expression *in planta* of a membrane transporter CzcCBA, a member of the Czc family (cobalt/zinc/cadmium) efflux transporters. This system is a trans-envelope pump, typical of gram-negative bacteria, and acts as a chemiosmotic antiporter. The CzcCBA complex is constituted of three components: A, B and C. The first is localized at the inner membrane of the bacterial cell and allows the efflux of metal cations from the cytosol to the periplasmic space. The B subunit is located in the periplasmic space and directs ions towards the third component, C - located in the outer membrane, which opens a channel extruding metal ions outside the bacterium.

Since these membrane transporters contribute to the resistance to high heavy metals concentration in *P. putida*, we tried to utilize them to modulate heavy metal accumulation in transgenic plants. Both *Arabidopsis thaliana* and *Nicotiana tabacum* were selected as model species. Firstly, to understand the localization of the three genes in the plant cell, *CzcA*, *CzcB* and *CzcC* were cloned into the pUC-35S::NosT plasmid, in fusion with the eGFP reporter gene, under the control of the CaMV 35S promoter. Protoplasts transient transfection highlighted a probable plasma-membrane and nuclear localization of CzcC, while for CzcA and CzcB results need to be confirmed. The three genes were also cloned into appropriate plant-overexpression vectors, and transferred into the plant genome via *Agrobacterium* transformation. Preliminary data on the expression of *CzcA* and *CzcB* in tobacco, show that these genes alone are able to modulate cadmium amounts absorbed by transgenic plants, when compared to wild type control. In particular, transgenic plants overexpressing CzcA accumulate lower cadmium amounts in comparison to the wild-type.

Considering that the Czc system is constituted of three subunits, transgenic plants harboring the three single Czc genes are being crossed, to isolate individuals that carry the complete CzcCBA complex and to verify whether the entire transporter confer different cadmium transport ability to plants.