

## **IDENTIFICATION OF GENES AND PROTEINS INDUCED BY HEAVY METALS IN HYPERACCUMULATOR PLANTS UNDER THE INFLUENCE OF RHIZOBACTERIA**

FARINATI S., CORBELLA M., DAL CORSO G., FURINI A.

Department of Science and Technology, University of Verona, Strada Le Grazie 15, 37134 Verona (Italy)

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Environmental pollution by heavy metals is a relevant global problem which has its negative impact on human health and agriculture. However, the identification of several plant species that can tolerate and accumulate high concentrations of metals in their above-ground tissues, suggests that the genetic potential for phytoextraction exists. Therefore the development of phytoremediation technologies for cleaning-up polluted soils by heavy metals is of significant interest. Unfortunately, our knowledge about the molecular mechanisms responsible for phytoextraction is limited. Hence, an aspect of our research is focused on the characterization of genes involved in metal uptake, transport to and accumulation in the above-ground tissues. For instance, the study of BjCdR15, a bZIP transcription factor identified in *B. juncea* after Cd treatments, demonstrated that this gene plays a significant role in long distance transport from root to shoot. Furthermore, it is well known that in phytoremediation process of contaminated soils by heavy metals it is important to consider the influence of the rhizosphere: in fact, it is worth to note that the microbial community of rhizosphere can positively affect both metal uptake and plant growth. On the other hand, plant may increase the growth and the metabolic activity of these microorganisms.

The aim of our research is to molecular characterisation the interaction between plants and microorganisms during the metal uptake process. Particularly we focused on the identification and characterization of putative candidate genes involved in tolerance/hyperaccumulation mechanisms in the presence of rhizobacteria. We considered an ecotype of *A. halleri* (a well-known cadmium- and zinc-hyperaccumulator) adapted to grow in a contaminated soil by Pb, Cd and Zn. Plants were cultivated in Hoagland's solution and, after a period of acclimation, they underwent to four different growth conditions: (1) in Hoagland nutritive solution (control), (2) with the addition of heavy metals (1 mM Cd and 10 mM Zn) to Hoagland's solution, (3) with the addition of the same heavy metals and the microbial community of the *A. halleri* rhizosphere and (4) in the solely presence of the microbial community of the *A. halleri* rhizosphere added to nutrient solution. After one month the total Cd and Zn content in roots and shoots of plants subjected to different treatments was determined. In the presence of rhizobacteria, Cd and Zn contents were higher in above-ground tissues respect to roots (trial 3). In addition, in these plants, the heavy metal content was higher in vegetative tissue when they were inoculated with the microbial community of the *A. halleri* rhizosphere compared to plants grown with the solely addition of heavy metals (trial 2). These results confirmed that microbial rhizosphere community positively affect the uptake and the root-to-shoot transport of heavy metals in *A. halleri*. Furthermore, we have identified proteins differentially expressed in shoots of plants treated with heavy metals or heavy metals plus rhizobacteria applying

a proteomic approach (2D-PAGE) aimed to recognize main genes involved in heavy metal tolerance, transport and hyperaccumulation modulated by the interaction plant-microorganisms. 61 differentially expressed proteins were classified based on their biological function and cellular localization: most of them are involved in cellular metabolism, photosynthesis, photorespiration and biotic/abiotic stress response. In conclusion, this work will allow further characterization of genes responsible for Cd and Zn tolerance and hyperaccumulation, suitable for biotechnological applications in phytoremediation approaches.