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PROTEOMIC AND EXOPROTEOMIC ANALYSIS OF ACTJK-DRIVEN RESPONSES TO COPPER AND ZINC STRESS IN *Sinorhizobium meliloti*

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Rhizobia-legume symbiotic interaction implies severe physiological changes in bacteria from the free-living state in the soil to the oxygen-limiting environment of plant root nodules. To tolerate and adapt to changing and stressful environments, bacteria modify their physiology as a defense mechanism. We recently described the two-component system ActJK present in *Sinorhizobium meliloti*, which is involved in the response to acid stress adaptation and during the symbiotic process with legumes. In natural environments with low pH the challenges faced by bacteria are not only due to higher concentrations of H⁺, but also to changes in the availability of other ions due to soil acidity. Indeed, we found that ActJK is also required for Cu and Zn stress response. Thus, to investigate the molecular basis and the role of ActJ on the biology of *S. meliloti* we performed proteomic and exoproteomic analysis under Cu or Zn stress.

Four replicates of *S. meliloti* (wt) and Δ actJ were cultured in GSM pH 7.0 until log phase. Then, the cultures were supplemented with either CuSO₄ or ZnSO₄ or without any stressor and grown for 2 hours. Cells were then centrifuged at max. speed. The pellets were used to extract membrane and cytosolic proteins, while filtered supernatants were used for exoproteins. Peptides were analyzed using a hybrid quadrupole mass spectrometer Orbitrap Q Exactive Plus LC-MS/MS connected to an UltiMate 3000 HPLC. A Data-Independent Acquisition approach was employed to identify the peptides using Proteome Discoverer. Perseus was used to process the raw data and analyze the results.

The proteomic analysis identified a total of 3566 peptides in the samples. Principal component analysis for the replicates indicates that the control and excess Zn conditions are more closely related to each other than to the excess Cu condition. Differential proteins were defined as those with Δ actJ (on) or the wt (off) were analyzed. We compared upregulated or downregulated proteins. Although each condition has numerous differential proteins, few proteins are shared between the treatments and many of them have not been characterized. However, DegP1 and MacAB were found to be downregulated in Δ actJ under Cu and Zn stress. These proteins are also involved in the ActJK-mediated response under acid stress, highlighting the link between acidity and metal sensitivity. The exoproteomic experiment identified a total of 1164 peptides. PCA analysis

showed a similar distribution among samples and conditions compared to the proteomic experiment. DegP1 was also found to be downregulated in $\Delta actJ$ /wt under Cu and Zn stress.

Our findings show that while ActJK-mediated responses to Cu or Zn involve distinct pathways or cascades, a few key components of the signaling cascades are interconnected. Furthermore, they share common nodes with proteins like MacAB and DegP1, which are also involved in the acid stress response.

Palabras clave: Metal stress – Two-Component System – Proteomic – Exoproteomic – Signal transduction