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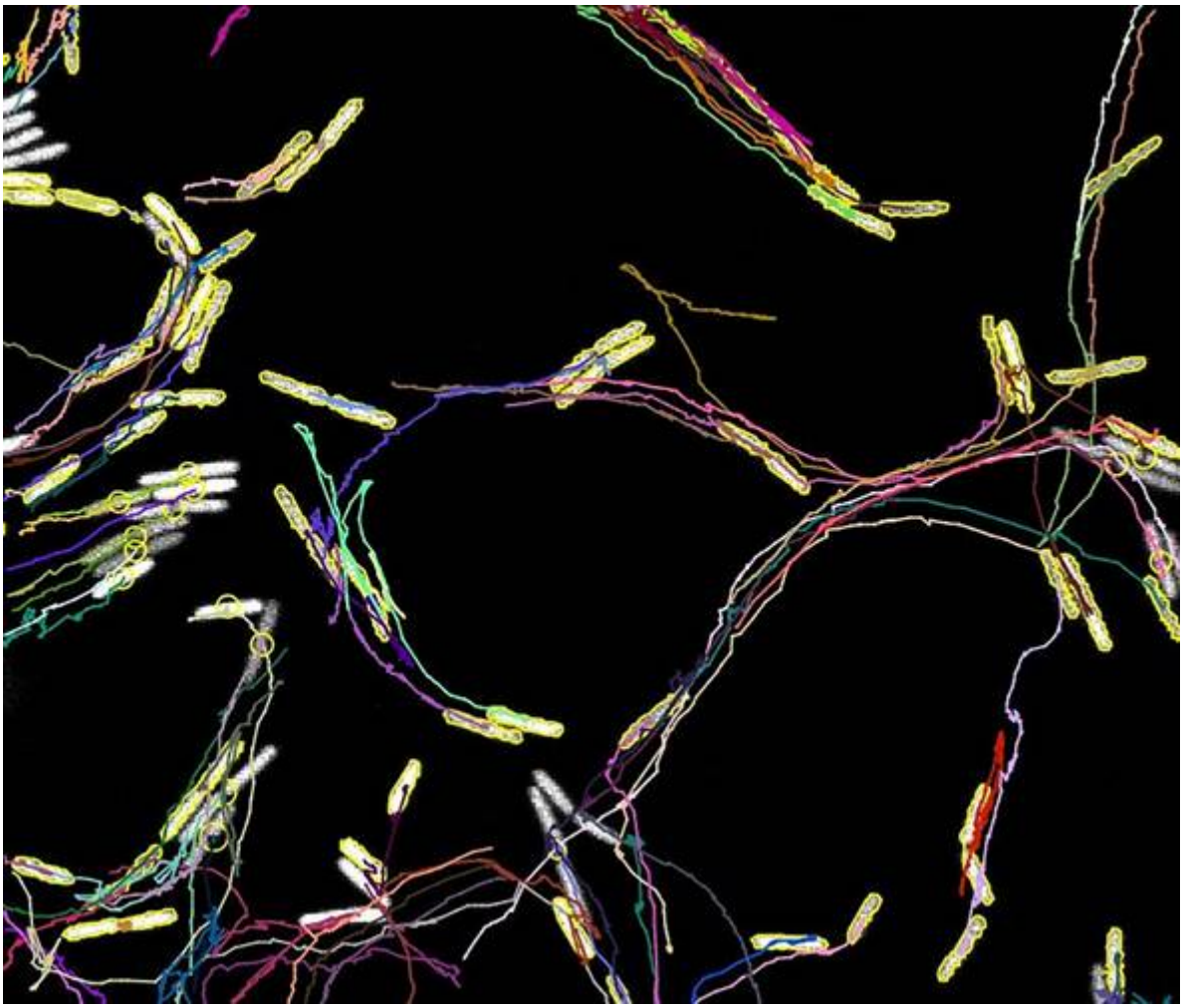

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ROLE OF ActJK TWO-COMPONENT SYSTEM IN COPPER AND ZINC HOMEOSTASIS IN Sinorhizobium meliloti

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Rhizobia are soil bacteria that form symbiotic relationships with legumes, enabling biological nitrogen fixation by converting atmospheric nitrogen into ammonium. This process is vital in agriculture, providing a sustainable alternative to nitrogen fertilizers. Our research centers on the symbiosis between *Sinorhizobium meliloti* 2011 and *Medicago sativa* (alfalfa), which is negatively impacted by acidic soils. In such conditions, the high concentration of H? ions and the altered bioavailability of metals like copper and zinc challenge the bacteria, the plant, and the symbiotic process, negatively impacting ecosystem biodiversity and productivity. Bacterial two-component systems (TCS) are versatile signaling mechanisms that govern cellular responses to diverse environmental signals. ActJK is a TCS of *S. meliloti* that plays an important role in acid tolerance and symbiosis with alfalfa. Our research has deepened the understanding of the role of ActJK, especially regarding copper and zinc stress. We examined whether the deletion of ActJ response regulator (?*actJ*) affects the growth of *S. meliloti* in a defined medium (GS) supplemented with micromolar concentrations of copper or zinc. The ?*actJ* strain showed reduced growth compared to the wild-type (wt) under these conditions. To explore the connection of ActJK with genes involved in copper and zinc homeostasis, a plasmid-based bacterial with eGFP transcriptional fusion reporter was used to measure the promoter activity of the genes encoding some selected metal exporters: copA1a, copA1b, and zntA. The results showed that those promoter activities in the presence of Cu? or Zn^{2}? are independent of ActJ. We performed comparative proteomics to find determinants linking ActJK with copper and zinc homeostasis. These omic analyses identified proteins with different functions with altered expression in wt strain compared to ?actJ strain under optimal growth conditions (GS medium, pH 7.0) and elevated concentrations of copper or zinc. The involvement of identified proteins in metal tolerance was further validated by testing the growth rate under copper or zinc metal stress in selected isogenic mutants. This revealed that the absence of Smc02363, a gene upregulated in ? actJ/wt under zinc excess, led to reduced growth compared to the control strain when exposed to zinc. Additionally, other transcriptional fusions were used to investigate the role of ActJ in *S. meliloti*. Our experiments demonstrated that ActJ regulates the expression of DegP1 (a chaperone-protease), MacAB (an efflux pump), and Smc02220 (a protein of unknown function) in response to copper and zinc stress. These findings offer insights into the stress response pathways

of *S. meliloti*, suggesting that ActJ plays a crucial role in regulating key genes involved in metal homeostasis and highlights its importance in the survival of *S. meliloti* in challenging environments.

Palabras clave: Sinorhizobium meliloti - Two component systems - ActJK - Metal stress.