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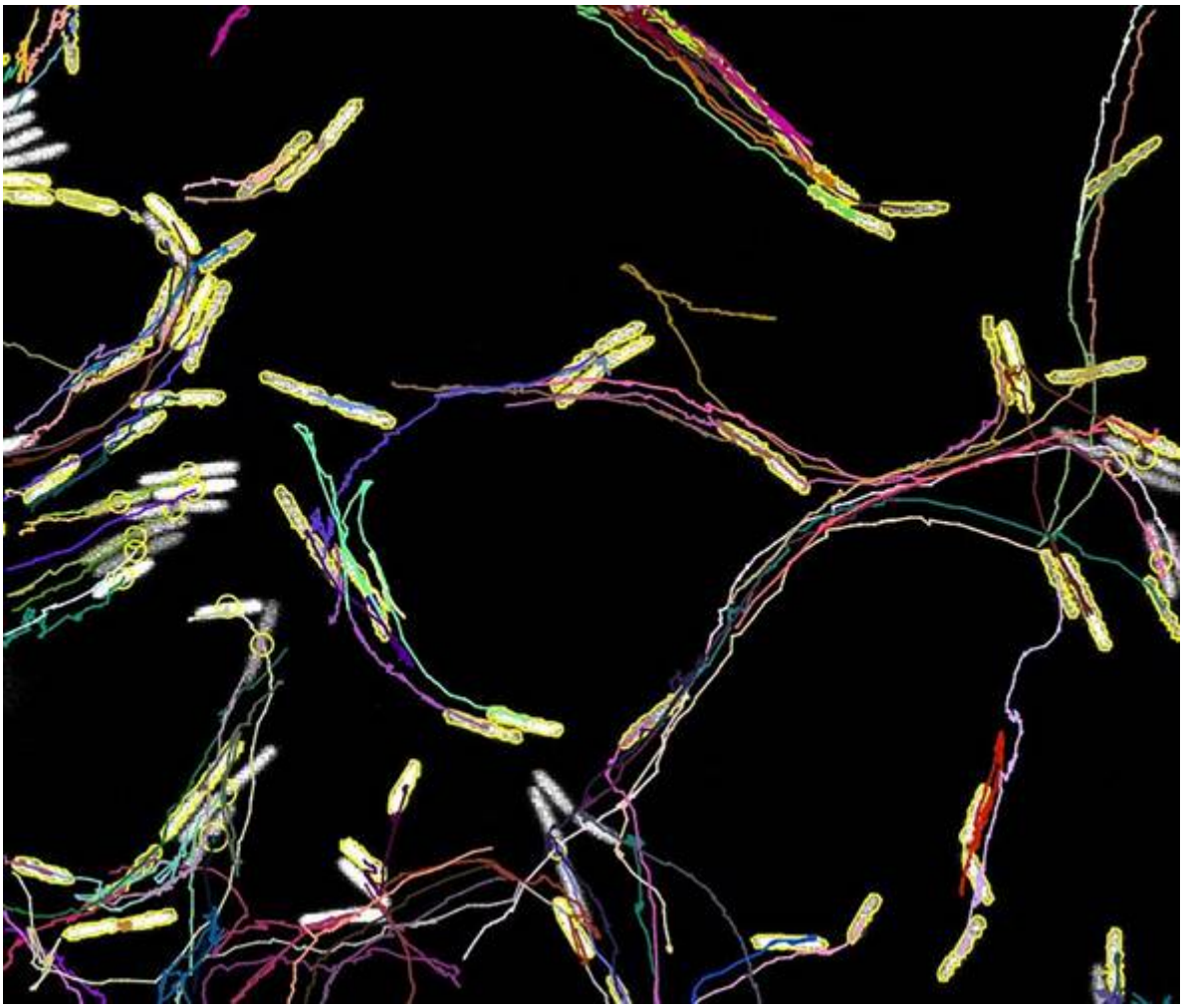

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

EXPLORING THE FUNCTION OF EXSG/F IN Sinorhizobium meliloti THROUGH IN SILICO AND IN VIVO ANALYSIS

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Sinorhizobium meliloti is a soil bacterium that can establish nitrogen-fixing symbiosis with the legumes *Medicago*, *Mellilotus* and *Trigonella*. The legume-rhizobia symbiosis is one of nature's most well-characterized mutualistic interactions, which plays an increasingly important role in sustainable agriculture. However, environmental stresses are limiting factors for an effective nitrogen-fixing symbiosis. In bacteria, several important biological processes are controlled by two-component systems (TCSs). The most simplified scheme of a TCS consists of regulatory pairs of one sensor histidine kinase (HK), which senses environmental changes, and one response regulator (RR), which activates genes to surpass the disruption. ExsG/F (HK/RR) is one of the TCSs present in *S. meliloti* that could be involved in the response to heat and starvation, as previously observed. In this work, we carried out the characterization of this TCS. Different bioinformatics tools used for in silico characterization (BlastP, Clinker, MEGA, among others) showed that these proteins are contiguously encoded and contain typical domain architectures and amino acid residue conservation. The analysis of gene neighborhood revealed synteny within Rhizobiales order. In addition, the phylogenetic trees generated for ExsG and ExsF suggested a conserved evolution between the two proteins. To experimentally confirm the operon prediction, we performed intergenic PCR reactions on randomly synthesized cDNA to analyze whether both genes were co-transcribed in a unique mRNA. Results showed that in both tested conditions (28°C and 42°C) *exsG* and *exsF* were co-transcribed in a unique mRNA in *S. meliloti*. Additionally, we constructed isogenic mutants for the HK, the RR and both components. Then we evaluated the free-living phenotype under heat stress. We performed growth curves for *S. meliloti* 2011 wild-type (wt), and single or double deletion mutants in *exsG* (HK) and/or *exsF* (RR), in rich (TY and LB) and minimal (SG) media at optimal temperature (28°C) or elevated temperatures (37°C and 40°C). The results revealed that the deletion of exsF and/or *exsG* did not significantly affect the growth rate under the evaluated conditions. These results suggest that both ExsF and ExsG proteins might play a role under different abiotic stresses, both in free-living conditions and in symbiosis. Currently, we are evaluating other possible phenotypes in which ExsG could be involved.

Palabras clave: Sinorhizobium meliloti – Two component system – Response regulator – Histidine kinase