Sociedad Argentina de Microbiología General

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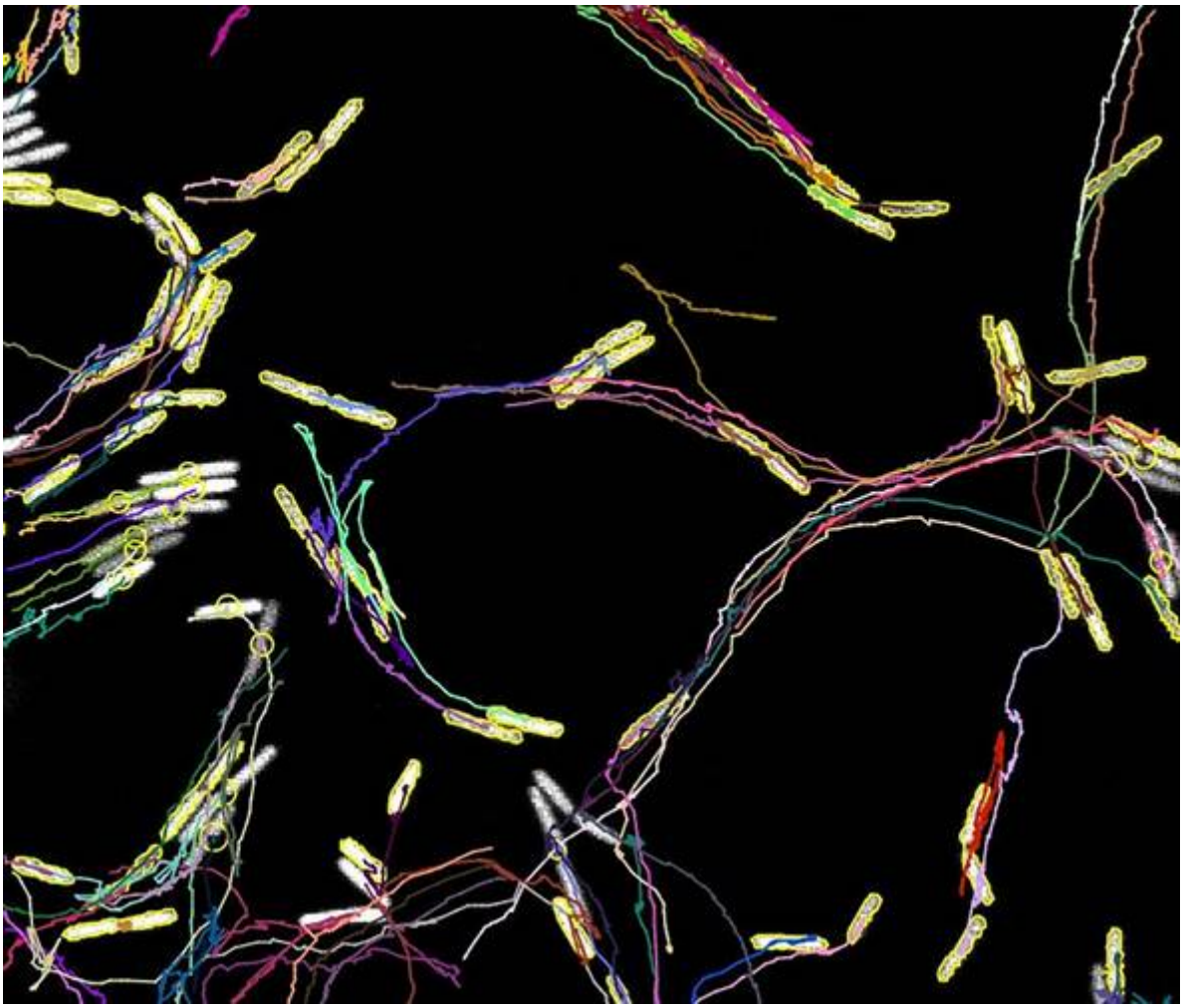

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

MOLECULAR CHARACTERIZATION OF Bradyrhizobium diazoefficiens TRANSCRIPTIONAL REGULATOR (PhaR) PROTEIN

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Bradyrhizobium diazoefficiens is a soil bacterium that can live within soybean root nodules and under free-living conditions. It accumulates polyhydroxybutyrate (PHB) in both states, with the PhaR protein being a key regulator for PHB metabolism. Previous transcriptomic and proteomic studies of a *phaR* mutant compared to the wild type, both grown under microaerobic conditions with mannitol, showed that PhaR has a pleiotropic function and regulates not only PHB metabolism, but also central carbon and nitrogen allocation pathways, as well as universal stress and motility proteins. Interestingly, PhaR also modulates the microaerobic-responsive regulatory network by activating the expression of fixK 2 and repressing nifA, both encoding two transcription factors relevant for microaerobic lifestyle. In this study, we applied a multidisciplinary approach to dissect the molecular mechanism of the PhaR regulator, including an in silico DNA motif prediction, analysis of its oligomeric state, and PhaR-DNA interaction assays. We then identified two conserved PhaR binding motifs (PhaR box): a 12-bp regular pattern containing a conserved GCx(3)GC sequence present at single or multiple locations within the promoter region of target genes, and a novel, alternative, and longer 22-bp pattern also enriched in G and C. Purified recombinant PhaR protein effectively interacted with either of PhaR box type, thus leading to the identification of novel 7 PhaR direct targets in addition to phaP1, the model target for PhaR, encoding one of the phasins of *B. diazoefficiens*. Interestingly, the functional mutagenesis of the *phaP1* promoter which harbors two regular patterns, revealed that both are important for PhaR interaction as tetramer and that the double GC sequence in tandem plays a key role in this interaction. These findings suggest that regulation mediated by PhaR appears to be complex and that other players may modulate the function of this regulator.

Palabras clave: Polyhydroxybutyrate-PhaR protein-DNA-protein interactions