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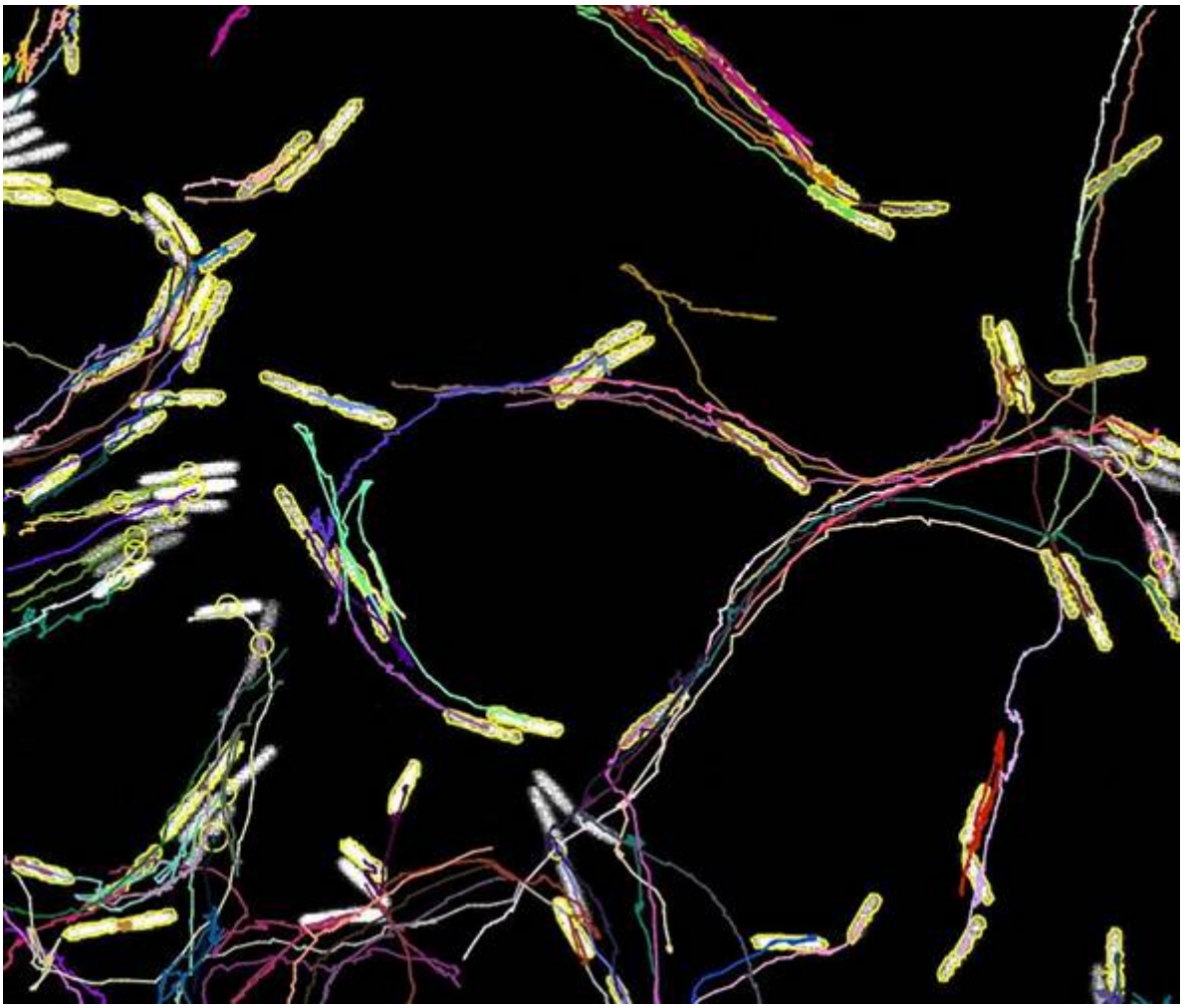

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

FUNCTIONAL ASSESSMENT OF A DIGUANYLATE CYCLASE-CONTROLLING PATHWAY FROM Halomonas titanicae KHS3 IN A HETEROLOGOUS ENVIRONMENT

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Halomonas titanicae KHS3 is an environmental bacterium isolated from the Argentine Sea that has a chemosensory signaling pathway, HtChe2, which controls the activity of a diguanylate ciclase (Ht-DGC). Constitutive activation of the pathway results in colony morphology alterations and increased biofilm formation. Such characteristics resemble the behavior of *Pseudomonas* Wsp (wrinkly spreader) chemosensory system, whose activation triggers the production of cyclic di-GMP and concatenates the switch to biofilm lifestyle. In this work, we investigated functional aspects of the HtChe2 pathway in a heterologous context using assays based on biofilm formation at the air-liquid interface (pellicles). On one hand, we analyzed the in vivo specificity of Htc10, the pathway chemoreceptor. We have recently determined the crystal structure of Htc10 ligand binding domain bound to guanine and hypoxanthine and identified four crucial amino acids involved in stabilizing the protein-ligand complex. To test whether the chemoreceptor responds to its specific ligands in vivo, we expressed Htc10 in *Pseudomonas putida* KT2440 and in different knockouts of the Wsp pathway, and analyzed the biofilm phenotype. Results shown that heterologous expression of Htc10 in a mutant strain lacking the native Wsp chemoreceptor (?*wspA*) promoted biofilm formation, a phenotype that was further enhanced by Htc10-specific ligands, guanine and hypoxanthine. When the assay was repeated in a mutant strain lacking the histidine kinase WspE the mentioned phenotype was abolished, corroborating that the full pathway needs to be present and active to trigger biofilm formation. An Htc10 variant with replacements in the amino acids involved in ligand binding was tested in the wild type, ?*wspA* and ?*wspE* backgrounds. Whereas biofilm formation was unexpectedly high in wild type and ?*wspA* strains, it was very low in the absence of the kinase, indicating again the dependence on the pathway. In no case differences between pellicle formation in the presence or absence of the ligands were observed. On the other hand, we aimed to understand HtChe2 diguanylate cyclase activation. Ht-DGC contains a phosphotransfer domain, followed by two response regulator domains in its amino terminus. By site directed mutagenesis we replaced the two predicted phosphorylatable aspartates with alanine and tested the ability of the mutant protein to induce biofilm formation in the wild type and ?*wspR* mutant strain (knockout of *P. putida* DGC). Quantitative analysis of the effects of Ht-DGC variants showed that increases in pellicle formation was associated with having at least one of the aspartates. This

is the first description of binding specificity of a chemoreceptor that controls the activity of an associated diguanylate cyclase, opening the way for dynamic studies of the signaling behavior of this kind of sensory complex.

Palabras clave: WSP - diguanilato cyclase - biofilm - Halomonas titanicae