IDENTIFICATION OF CRITICAL NUCLEOTIDES FOR THE INTERACTION BETWEEN THE QUORUM REGULATOR CerM AND ITS BINDING SITE IN *Cereibacter sphaeroides*

Dana S. Arana Hidalgo, Hernández Valle José, Laura Camarena.
Department of Molecular Biology and Biotechnology, Biomedical Research Institute. Circuito, Mario de La Cueva S/N, University City, Coyoacán, 04510, Mexico City, Mexico. Tel. 5556229222.
E-mail: rosal@gmail.com

The quorum sensing system (QS) is a cellular signaling network that allows bacteria to regulate gene expression as a function of their cell density. Typically, in this system there is a signal molecule called an autoinducer that is synthesized and continuously secreted into the extracellular space. Once it reaches a critical threshold concentration, the autoinducer is recognized by a transcriptional regulator, activating intracellular signaling pathways that orchestrate collective responses[1]. An important trait controlled by the QS is flagellar motility. The α-proteobacterium *Cereibacter sphaeroides* carries two different flagellar systems of distinct phylogenetic origins that are controlled by independent sets of regulatory proteins; the flagellar *fla1* and *fla2* genes. Expression of the *fla2* genes is dependent on the activation of the two-component system (TCS) CckA/ChpT/CtrA.[2,3] In many species, activation of the QS response has been found to regulate *ctrA* expression indirectly, through an unknown intermediate.

The QS of *C. sphaeroides* consists of a single autoinducer synthase and six transcriptional regulators homologous to LuxR. The LuxR-regulator, named CerM regulates the expression of approximately 181 genes, among which *xrpA* is significant, as it encodes a repressor of *ctrA*. From the RNAseq data, the CerM binding was proposed; however, this site is not well conserved[4]. Therefore, we hypothesized that the CerM recognition site might contain a small number of specific bases that could be essential for binding.

To identify the nucleotides that could be indispensable for CerM binding to its target site, we obtained site-directed mutagenesis substitutions in nucleotides located in the putative CerM binding site. Our results show that at least 3 of the nucleotides contained in the MBS are required for CerM binding.

[1]Miller MB, Bassler BL. (2001). *Annu Rev Microbiol*.;55:165-99,
[2]Camarena, L., Dreyfus, G. (2020). *Biomolecules*, 10(5), 774,
[3]Rivera-Osorio, A. *et al*. (2018). *BMC Microbiol* 18, 129,
[4]Hernández-Valle J, *et al*. (2024) *Microbiologyopen*;13(6):e012.