Poster

Study of the expression of *ecfG1* and *ecfG2*, two extracytoplasmic function sigma factors (ECFs) in *Sphingopyxis granuli* TFA.

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ABSTRACT

Motivation: In bacteria, the initiation of transcription requires a specific multi-domain subunits of RNA polymerase (RNAP) called sigma (σ) factors that binds to its core that play critical roles, including the recognition and opening of promoters for the RNA synthesis (Paget, 2015). One type of sigma factors are extracytoplasmic function sigma factors (ECF) which provide a means of regulating gene expression in response to a wide range of environmental changes (Feklistov et al., 2014). *Sphingopyxis granuli* TFA is a Gram-negative Alphaproteobacteria that is one of the few strains able to grow on the organic solvent tetralin as a sole carbon and energy source and able to grow respiring nitrate under anaerobic conditions (Gonzalez-Flores et al., 2016). In *Sphingopyxis granuli* TFA two ECF σ factors have been described, EcfG1 and EcfG2, that have a critcal biological role in the General Stress Response (GSR) in this bacterium (de Dios et al., 2020)

Methods: With the aim of studying the transcriptional and postranscriptional regulation of each *ecfG* genes, a recombinant protein was built in which each EcfG have a FLAG-tag fused which allowed us to quantified the amount of each sigma factor by Western Blot studies in different growth conditions. This protein was constructed using a DNA-recombination method based on a double-strand break caused by SceI nuclease. Flanking regions of each *ecfG* genes were cloned in a multiple cloning site (MCS) of a non-replicative vector, this MCS is flanked by two SceI target sites. When this integrative vector is integrated into the chromosome of TFA, a broad host range vector including SceI gene downstream of an inducible promoter must be introduced and a double-strand break is caused in the chromosome. The final repair of this break results with a high frequency in the deletion of the target gene (*ecfG*). Thus we only get the gene with the FLAG-tag

Results: In this work we analyzed the levels of expression of each sigma factors both at the transcriptional by RNA-seq and at translational level by quantifying the levels of each protein through Western Blot studies under different growth conditions

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