STUDY OF THE EXPRESSION OF *ECFG1* AND *ECFG2*, TWO EXTRACYTOPLASMIC FUNCTION SIGMA FACTORS (ECFs) IN *SPHINGOPYXIS GRANULI* TFA

Rosario Elena Armijo Miranda, Rubén De Dios Barranco and Francisca Reyes-Ramírez

Departamento de Biología Molecular e Ingeniería Bioquímica, Centro Andaluz de Biología del Desarrollo (CABD/CSIC), Universidad Pablo de Olavide, ES-41013 Seville, Spain.

INTRODUCTION

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 and 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 critical biological role in the General Stress Response (GSR) in this bacterium (de Dios et al., 2020) (**Figure 1**).

Figure 1. Genetic organization of the genes related to the GSR in the *S. granuli* TFA genome. The gene cluster is made up of tree main regulatory elements: an EcfG-type ECF σ factor, its cognate anti-σ factor NepR and the response regulator PhyR.

RESULTS

1- Construction of EcfG1::FLAG

With the aim of studying the transcriptional and postranscriptional regulation of *ecfG1* gene, a recombinant protein was built with a FLAG-tag fused. This protein was constructed using a DNA-recombination method based on a double-strand break caused by SceI nuclease (**Figure 2**).

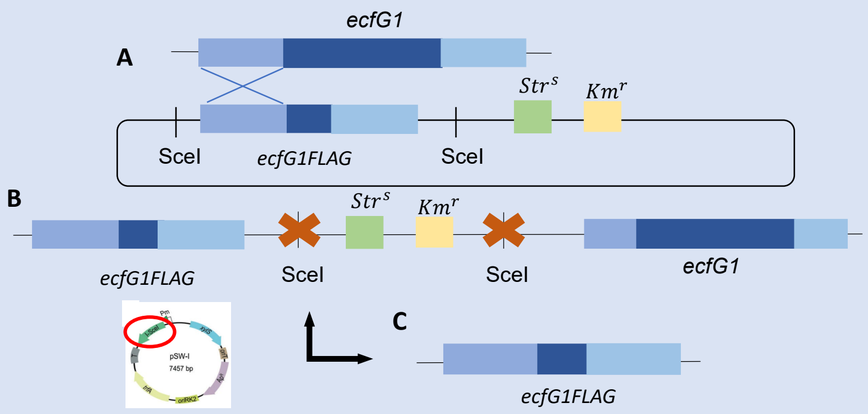

Figure 2. Construction of TFA strain with EcfG1 fused to a FLAG-tag. The first recombination between non-replicative vector and chromosome would occur between an EcfG1 flanking sequence and its homologous region (A). The plasmid would be integrated into the chromosome (B) and a second forced recombination would produce based on a double-strand break caused by SceI targets which would delete the wild type *ecfG1* gene (C).

2- Levels of EcfG1 protein measured by Western Blot studies under different growth conditions

Our research group carried out previous tests in which the levels of expression of each sigma factors were analyzed at the transcriptional level both by RNA-seq and B-galactosidase activity under different conditions. With the RNA-seq assays, the amount of transcripts under anaerobic conditions was higher than in aerobic conditions, however, the results obtained with B-galactosidase activity assays were completely contrary.

These results were contradicted each other, therefore, we analyzed its expression at translational level by quantifying the levels of EcfG1 through Western Blot studies under different conditions too. **Figure 3** shows the levels of EcfG1 protein obtained loading different amounts of cells; these results agree with those obtained by RNA-seq studies since the levels of EcfG1 under anaerobic conditions were higher.

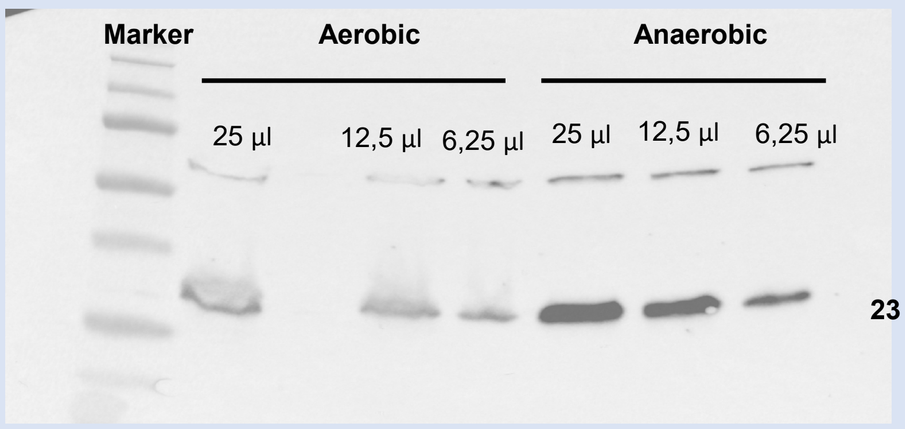

Figure 3. Membrane resulting from the Western Blot assay for study the levels of EcfG1 protein under different conditions. Decreasing amounts of cells were loaded onto the acrylamide gel.

3-TFA wt and ΔHfq growth curves

Hfq is a bacterial RNA chaperone that facilitates the binding of the sRNA to its target and protects it from degradation, therefore, is involved in the translational regulation of many different bacterial pathways (García-Romero et al., 2018). Due to its importance, the growth of wild type TFA and Hfq mutant was studied under anaerobic and aerobic conditions.

As seen in the **Figure 4**, there is no remarkable difference between wild type TFA with Hfq mutant. With these results two things can be concluded, on the one hand, the Hfq mutation wouldn't affect growth under these conditions. On the other hand, under aerobic conditions growth occurs exponentially and in less time than under anaerobic conditions even after a redilution of the anaerobic assay.

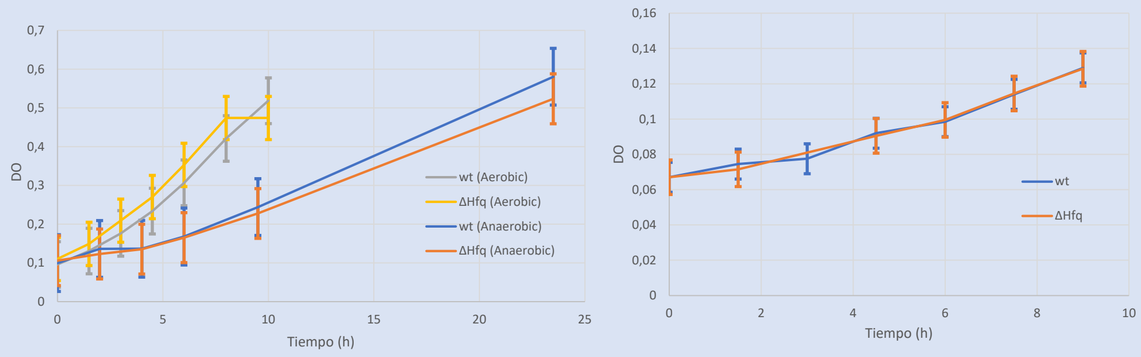

Figure 4. Growth curves of wild-type TFA and *Δhfq*. The first graph shows the growth curve of WT and *Δhfq* under aerobic and anaerobic conditions. The second graph shows the growth curve under aerobic condition of WT and *Δhfq* after a redilution of the anaerobic assay.

CONCLUSIONS

In this work, we obtained higher levels of EcfG1 protein under anaerobiosis than in aerobiosis, which would agree with the RNA-seq results. For this reason, the idea of the presence of postranscriptional regulatory mechanisms raised by the results obtained from B-galactosidase activity would be refuted. However, future assays in EcfG2 could further clarify the objectives of this work.

On the other hand, the possibility that a mutation in the Hfq gene affected to growth capacity under these given conditions would be also denied.

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