Poster

Role of the ECF sigma factor EcfG in the general stress response of *Sphingopyxis granuli* TFA.

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ABSTRACT

Motivation: One of the main mechanisms bacteria use to face ever-changing environmental conditions is the swapping of sigma factors. These are small proteins which direct the core RNA polymerase in its target promoter recognition (Staron et al., 2009). On the other hand, *Sphingopyxis granuli* TFA is an alphaproteobacterium capable of growing using the organic solvent tetralin (1,2,3,4-tetrahydronaphtalene) as a sole carbon source (Moreno-Ruiz et al., 2003). Preliminar RNA-seq data showed that the alternative sigma factor EcfG, a homolog of the general stress response master regulator in alphaproteobacteria, is strongly overexpressed in the presence of tetralin, among other conditions. In this project, an *ecfG* deletion mutant was constructed and its phenotype partially characterized, regarding its capability to respond to a variety of stresses and to activate the expression of tetralin degradation genes.

Methods: The *ecfG* deletion mutant was constructed using molecular biology techniques and adapting the *Sce*I-based genomes editing platform to TFA (Martínez-García & de Lorenzo, 2011). This method consists of two plasmids: one with a narrow host range origin of replication and a MCS flanked by two *Sce*I targets and other one with a broad host range origin of replication and the *Sce*I nuclease ORF downstream an inducible promoter. Flanking regions of the target gene must be cloned within the MCS of the first plasmid and its integration in the chromosome, selected. Upon introduction of the second plasmid and induction of the nuclease, 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* in our case).
A bioinformatic analysis of TFA promoter sequences was performed in order to search for putative EcfG-regulated genes and thus, testable phenotypes.
Growth inhibition assays were performed in the presence of different stress agents and different culture media, either in liquid culture or disk diffusion assays. EcfG and tetralin degradation genes expression was measured by means of β-galactosidase activity assays using a lacZ translational fusion to *ecfG* and *thnC* promoters.

Results: The *ecfG* deletion mutant was not affected either in the ability to use tetralin as carbon source nor in the capability to activate the expression of the tetralin degradation genes compared to the wild type.
The mutant showed a slightly increased sensitivity to certain oxidative agents and antibiotics. However, it did not show a different behavior from the wild type strain in the presence of other compounds predicted to trigger the general stress response in this bacterium, according to the bioinformatic analysis, such as copper and fusaric acid.
In the case of its expression pattern, the *ecfG* mutant showed different activity levels compared with the wild type in all the conditions tested, such as presence of tetralin, stationary phase and anaerobiosis.

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