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Poster

Characterization of flagellar-specific sigma factor FliA in *Sphingopyxis granuli* TFA

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Keywords: sigma factor; flagella; *Sphingopyxis granuli* TFA.

ABSTRACT

Motivation:

Sigma factors are RNA polymerase subunits which play a crucial role in the transcriptional regulation of bacterial gene expression. These dissociable proteins bind to RNA polymerase controlling promoter recognition and thus, gene expression (Francez-charlot et al., 2015). Previous studies in *Alphaproteobacteria* identified a sigma factor FliA as a key transcriptional regulator of chemotaxis and flagella biogenesis by controlling the expression of genes involved in flagellin biosynthesis, an essential structural protein which forms flagella filament (Maruyama et al., 2015). *Sphingopyxis granuli* TFA is a Gram-Negative *Alphaproteobacteria* very interesting since 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 (García-Romero et al., 2016). The aim of this study is to construct and phenotypically characterize a *fliA* deletion mutant that help us to elucidate the function of FliA sigma factor and thus, the hierarchy of transcriptional regulation of flagellar genes, in *Sphingopyxis granuli* TFA.

Methods:

A *fliA* deletion mutant is being constructed using a DNA-recombination method based on a double-strand break caused by SceI nuclease. Firstly, flanking regions of *fliA* gene must be cloned in a multiple cloning site (MCS) of a non-replicative vector. At the same time, this MCS is flanked by two SceI target sites. Once this integrative vector is integrated into the chromosome and selected in wild-type TFA, a broad host range vector including SceI gene downstream of an inducible promoter must be introduced. A double-strand break is caused in the chromosome after the induction of SceI nuclease by 3-methylbenzoate and DNA will be repaired causing FliA gene deletion.

Once *fliA* deletion mutant is constructed, a phenotypic characterization will be performed. In addition, both semi-quantitative PCR and Q-PCR assays will be used in order to confirm which genes are regulated by FliA in *Sphingopyxis granuli* TFA.

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