Role of FnrS small regulatory RNA in the anaerobic response of Sphingopyxis granuli TFA.

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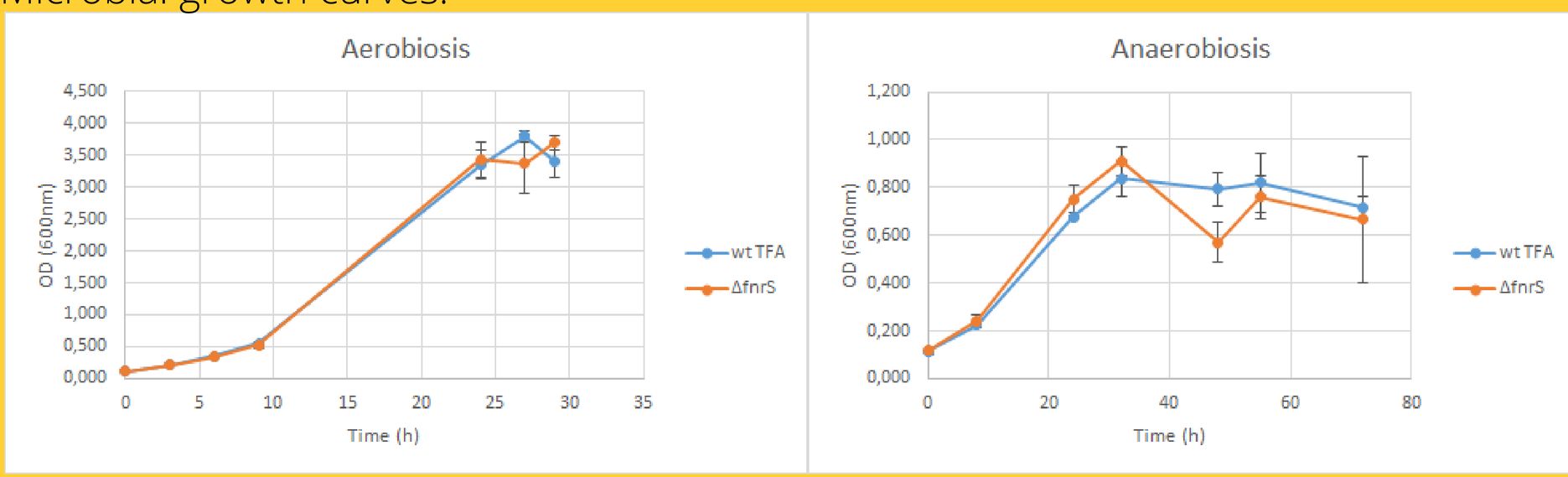
INTRODUCTION

Sphingopyxis granuli strain TFA is a Gram-negative α-Proteobacteria, that belongs to the *Sphigomonadaceae* family and that was isolated from the Rhine river in Germany (Dorn, E. et al., 1974). TFA is the first of its genus which exhibits the capability to grow under anaerobic conditions using nitrate as a terminal electron acceptor (García-Romero, I. et al., 2016). Non-coding RNAs (ncRNA) are critical regulators of bacterial responses to changes in the environment and achieve refined regulation through base pairing with mRNAs, modulating their stability and/or translation. These potential ncRNAs and their interaction targets have been identified in TFA by RIL-seq. Among them, a sRNA called FnrS is of particular interest since it seems to be induced under anaerobic conditions.
The aim of this project is to perform a general characterisation of this sRNA, including its regulatory mechanism and its function under anaerobic conditions.

METHODS & RESULTS:

Microbial growth curves:



Both wt TFA and ΔfnrS are able to grow under aerobic and anaerobic conditions.
Both variants show similar generation time, about **four hours under aerobic conditions** and more than **ten hours in the absence of oxygen** (Figure 1).

Fig. 1. Microbial growth curves. OD(600nm) over time under aerobic and anaerobic conditions is shown. This culture was grown using β-hydroxybutirate as a sole carbon and energy source.

Gene expression regulation:

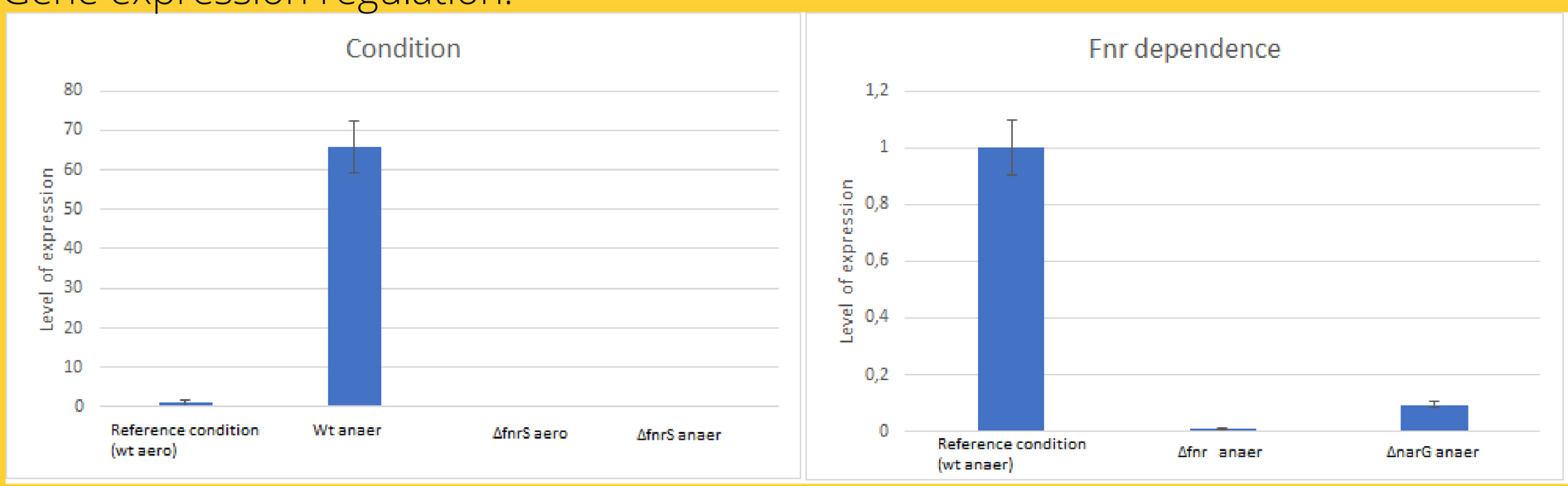


Fig. 2. Relative expression of the sRNA fnrS in TFA, ΔfnrS, Δfnr and ΔnarG.

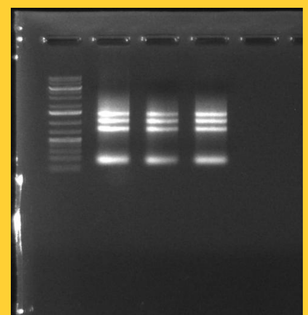
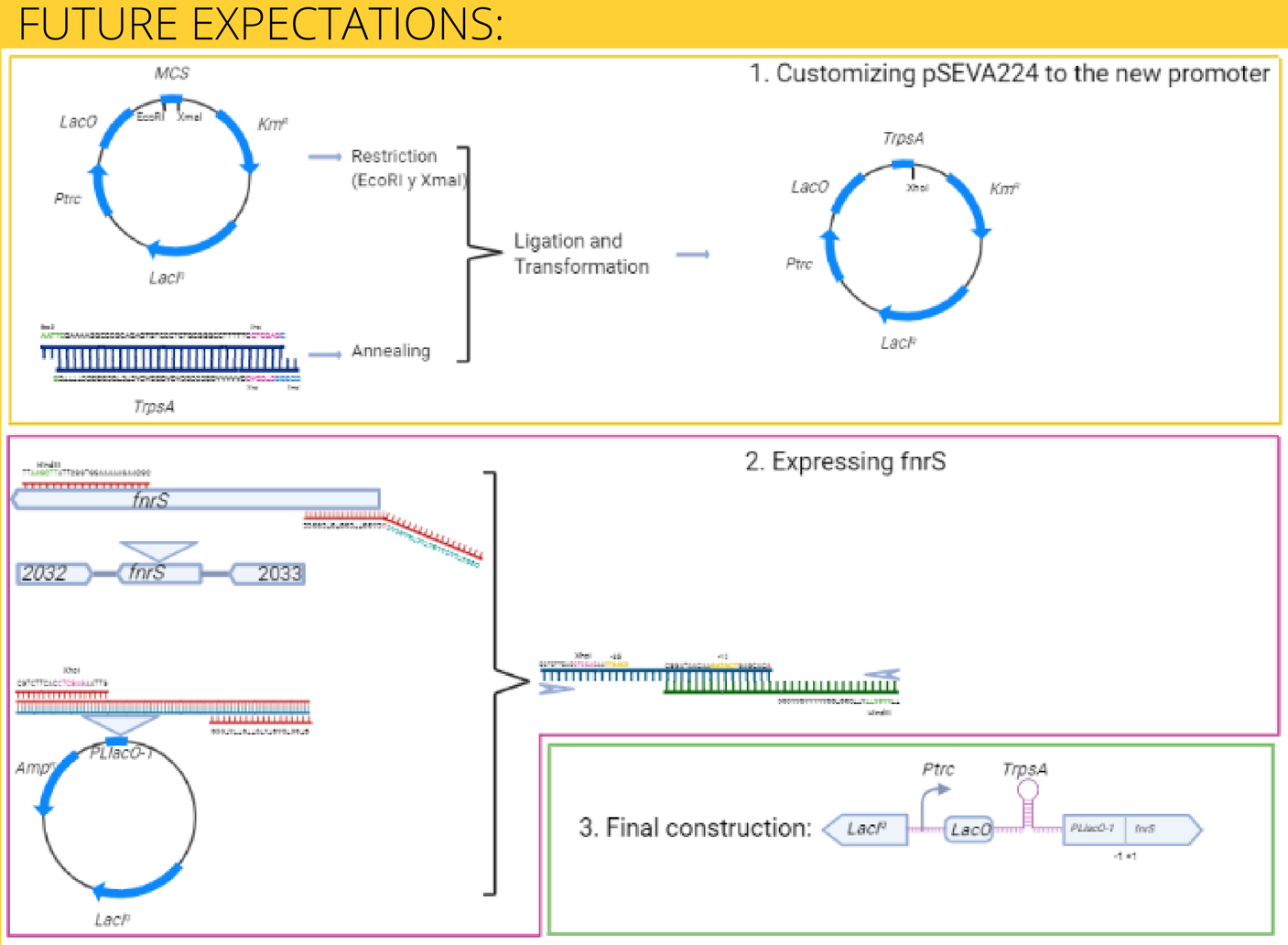


Fig. 3. Electrophoresis gel following to RNA extraction by phenol-cloroform method.

We compare by quantitative PCR the growth of the ΔfnrS vs. the wt TFA, in aerobiosis and anaerobiosis, and the dependence of the transcription of fnrS on Fnr proteins.
It is observed that fnrS is expressed *65.6 ± 6.4-fold more in anaerobiosis than in aerobiosis* in the wild-type strain. In ΔfnrS, the expression tends to zero.
It can be seen that fnrS is expressed *121.3 ± 1.0-fold less in anaerobiosis in the fnr double mutant* than in the wt TFA, and *11 ± 1.1-fold more in the ΔnarG mutant* than in the double mutant. This result indicates that **the transcription of fnrS is dependent on Fnr proteins and that regulation is altered, including growth**; in ΔnarG there is no growth either, but there is regulation of gene expression (Figure 2).
In Figure 3 we show an example of the RNA subsequent to its extraction with phenol-chloroform.

FUTURE EXPECTATIONS:



CONCLUSIONS:

The sRNA fnrS engages in anaerobic respiration but it is not essential for growth under this condition. FnrS is drastically more expressed under anaerobic condition, and its expression is conditioned by the regulatory proteins Fnr.

REFERENCES:

- Dorn, E., Hellwig, M., Reineke, W. and Knackmuss, H. J. (1974) Isolation and characterization of a 3-chlorobenzoate degrading pseudomonad. Arch Microbiol 99, 61-70.
- Garcia-Romero, I., Perez-Pulido, A.J., Gonzalez-Flores, Y.E., Reyes-Ramirez, F., Santero, E., Floriano, B. (2016) Genomic analysis of the nitrate-respiring Sphingopyxis granuli (formerly Sphingomonas macrogoltabida) strain TFA. BMC Genomics, 17, 1-15.

Pablo Pacheco thanks Francisca Reyes and Rubén de Dios for the experimental design, and Guadalupe Martín for the technical support.