Master 2 project 2021-2022

Characterization of methanol chimeric transcriptional regulators for synthetic methylotrophy in *E. coli*

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Description

In our team, we use synthetic and systems biology approaches to understand bacterial metabolism and reconstruct metabolic networks focusing on methylotrophy[1,2]. Methylotrophy is the capability of certain microorganisms to use reduced C1 compounds like methanol as their sole carbon and energy source. We have recently established a new synthetic methylotrophic pathway that allows methanol utilization in *Escherichia coli*[3]. Although this strain assimilates methanol into central metabolism molecules and biomass, it is still unable to grow on methanol as the only carbon source. Establishing utilisation of a non-native carbon source is a challenging task and one important bottleneck is that it requires pathway integration into the host's global cellular processes and transcriptional regulation is the link connecting nutrient metabolism with these other processes. As methanol is a non-native carbon source, one quesstion to be answered is: is it possible to make the regulatory network of *E. coli* respond to methanol?

In this Master project, we propose to address this question. To do so, the M2 student will engineer and characterize chimeric transcriptional regulators that combine a methanol-sensing domain with an *E. coli* DNA-binding domain. He/she will test the response to methanol of these fusion proteins using available high-throughput systems for cultivation and fluorescence monitoring. Depending on the results obtained and timing of project progress, promising chimeric regulators will be further evaluated on the basis of methanol utilisation using ^{13}C-labelling experiments.

Keywords

Methylotrophy, regulation, transcriptional regulator, synthetic biology.

Techniques

Bacterial cultures, molecular biology, high-throughput fluorescence assays, isotope-labelling experiments.

References

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[3] A. De Simone, C. M. Vicente, C. Peiro, L. Gales, F. Bellvert, B. Enjalbert, et al., *Metab. Eng.* **2020**.