OFFRE DE STAGE

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Titre du projet : In vivo and in vitro characterization of a native bacterial microcompartment system in *Escherichia coli*.

More than 20% of the sequenced bacterial genomes carry operons encoding for putative microcompartments (BMCs)[1]. BMCs are cytosolic organelles made of a proteinaceous shell that encapsulates specifically targeted enzymes. This spatial confinement drives metabolic channeling and allows sequestering highly toxic intermediates. BMCs thus act as intracellular nanobioreactors, with potential biotechnological applications[2]. Our team is interested in characterizing the native ethanolamine utilization (Eut) BMCs of *Escherichia coli*. Combining genome editing (CRISPR-Cas9 system), microbial physiology (growth, exometabolomic) and microscopy (epifluorescence, transmission electron microscopy) approaches, we recently showed that *E. coli* K12 is capable of producing functional Eut BMCs after induction of the *eut* operon. **Multiple aspects remain uncertain as to how the Eut BMCs form, how the enchased metabolism operates and what is the specific role of individual Eut proteins.** To start answering these questions, the selected student will help developing a protocol for the isolation of intact Eut BMCs. We will evaluate diverse methods relying on differential centrifugation steps, varying solubility as a function of salt concentration or protein tagging. We will assess the integrity of the isolated Eut BMCs by TEM, analyse their protein content by SDS-PAGE and evaluate the kinetics of the enchased reactions by ^{1}H-NMR. In parallel, we will knock out selected Eut proteins to evaluate the impact of the mutations on Eut BMCs function and integrity. This will be done both in vivo and in vitro with the newly established purification protocol. The generated knowledge will represent an important step forward to understand how a native BMC system functions and to design strategies to “hack” the BMC for the encapsulation of heterologous pathways of interest.

Techniques :
SDS-PAGE, Western Blot, ^{1}H-NMR, transmission electron microscopy, epifluorescence microscopy, CRISPR-Cas9, Plate reader

References :

1. Kerfeld, C. A., Aussignargues, C., Zarzycki, J., Cai, F. & Sutter, M. Bacterial microcompartments. *Nat. Rev. Microbiol.* **16**, 277 (2018).
2. Lee, M. J., Palmer, D. J. & Warren, M. J. Biotechnological advances in bacterial microcompartment technology. *Trends Biotechnol.* **37**, 325–336 (2019).