Grenoble Alliance for Integrated Structural & Cell Biology

INTERNSHIP PROPOSAL

Institute and Group: BIG, LBCI, PBRC team

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Research project title: Integrated study of a negative transcriptional regulator of bacterial virulence

5 Keywords to describe the project: *Pseudomonas aeruginosa*, host-pathogen interaction, repression, cupin domain, protein-DNA interaction

Description of the project (aims, experimental techniques, recommended background):

We previously described a new mechanism of virulence in the opportunistic human pathogen *Pseudomonas aeruginosa* that relies on the pore-forming toxin ExlA (Elsen *et al*., 2014; Reboud *et al*., 2016; Basso *et al*., 2017). To gets insights into toxin regulation, we performed a genome-wide transposon mutagenesis and identified a transcriptional repressor of *exlA*. We named it ErfA for "Exolysine regulatory factor A". The regulon of ErfA, as well as its conservation in other *Pseudomonas sp*., are currently being determined using next generation sequencing (NGS) technologies, such as RNA-seq and ChIP-seq. The proposed project will study structural and functional properties of ErfA using *in vitro* and *in vivo* approaches, such as DNA gel shift, site-directed mutagenesis and bacterial ExlA-dependent cytotoxicity. The role of ErfA's two predicted domains, the DNA-binding domain and the regulatory "Cupin" domain, will be evaluated *in vivo*. Conserved amino acids in the Cupin domain will be mutated to assess their effect on ErfA function and toxin expression.

Recommended background - bacteriology, molecular biology and biochemistry

Justification that the internship's subject fits with the general theme of GRAL:

The project aims at understanding structure-function relationship of a newly identified transcriptional regulator of bacterial virulence. The domains of the repressor will be studied *in vitro* and *in vivo*, in the context of a host-pathogen interaction/infection.

Relevant publications of the team:

1) **Elsen* S.**, Huber*, P., Bouillot, S., Couté, Y., Fournier, P., Dubois, Y., Timsit, J.F., Maurin, M., Attrée, I. **2014**. A type III secretion negative clinical strain of *Pseudomonas aeruginosa* employs a Two-Partner Secreted exolysin to induce hemorrhagic pneumonia. *Cell Host Microbe* 15,164-76. (*Contribution équivalente au travail).

2) Reboud E., **S. Elsen**, S. Bouillot, G. Golovkine, P. Basso, K. Jeannot, I. Attrée, P. Huber. **2016**. Phenotype and toxicity of the recently discovered exlA-positive *Pseudomonas aeruginosa* strains collected worldwide. *Environ Microbiol*. *18*, 3425-3439.

3) Basso P., M. Ragno, **S. Elsen**, E. Reboud, G. Golovkine, S. Bouillot, P. Huber , S. Lory, E. Faudry, I. Attrée . **2017**. *Pseudomonas aeruginosa* pore-forming exolysin and Type IV Pili cooperate to induce host cell lysis. *MBio*. 8(1).