**OFFRE DE STAGE**

**Laboratoire, équipe**: LMGM, Equipe Redder
**Encadrant**: Peter Redder, 0561335981, peter.redder@univ-tlse3.fr

**Titre du projet**: Organisation of the RNase J complex in *Staphylococcus aureus*

**Description du projet (2000 caractères max)** :
RNA degradation is a key element in gene regulation of all cellular organisms, and in bacteria the RNA decay is so efficient that mRNA molecules typically only have half-lives of 1-5 minutes.
The RNase J complex is composed of RNase J1 and RNase J2, and it degrades RNA from the 5'-end to become single nucleotides. This complex affects 80% of all *Staphylococcus aureus* RNA and is essential in under wide range of growth conditions, for example no growth at 42°C and 25°C (Linder et al. 2014). The goal of this M2 project will be to uncover how the interactions between RNase J1 and J2 are organised to ensure correct RNA degradation.

Part 1: Stoichiometry of the RNase J complex(es) in *S. aureus*.
The chromosomal genes for RNases J1 and J2 will be fused to two different immuno-detectable tags. A formaldehyde cross-linking will be carried out *in vivo*, which will covalently link everything that interacts in the bacterium (including the J1-J2 complex). A total protein extract will be separated on SDS gel. Two Western blots with the antibodies against the two different tags will make it possible to determine the stoichiometry of the J1 and J2 proteins for each form of complex (each band). This analysis will make it possible to determine the configuration(s) found *in vivo* (this strategy will also be applied to the RNase J mutants identified in Part 2).
Part 2: J1-J2 interaction surfaces
We have generated mutant strains where RNase J2 is expressed, but is incapable of forming interactions within the J1-J2 complex. The slow/no growth of these strains allow us to establish a genetic screening system to select for suppressor mutants that grow under restrictive conditions (e.g. 42°C). A pilot experiment has shown that such suppressor mutants form larger colonies on a petri dish. The student will isolate a range of suppressor mutant, and sequence the genes encoding RNases J1 and J2, in order to identify the suppressor mutations.

Briefly about the Redder group:
We study how RNA is degraded and how this process is organised and regulated in the human pathogen *Staphylococcus aureus*. The group is bi-lingual, with both French and English used.

**Techniques**:
Molecular cloning and vector construction.
Allelic replacement in *Staphylococcus aureus* (see Redder and Linder 2012).
Western blotting (quantitative).
Microbiological genetic screening (using *Staphylococcus aureus*).

**Références**:
Newman JA, Hewitt L, Rodrigues C, Solovyova A, Harwood CR, Lewis RJ. Unusual, dual endo- and exonuclease activity in the degradosome explained by crystal structure analysis of RNase J1. Structure. 2011 Sep 7;19(9):1241-51. doi: 10.1016/j.str.2011.06.017.
Linder P, Lemeille S, Redder P. Transcriptome-wide analyses of 5'-ends in RNase J mutants of a gram-positive pathogen reveal a role in RNA maturation, regulation and degradation. PLoS Genet. 2014 Feb 27;10(2):e1004207. doi: 10.1371/journal.pgen.1004207.
Redder P. Molecular and genetic interactions of the RNA degradation machineries in Firmicute bacteria. Wiley Interdiscip Rev RNA. 2018;9(2):10.1002/wrna.1460. doi:10.1002/wrna.1460