Title of the thesis project: **Functional redundancy within bacterial regulators: characterization of *Staphylococcus aureus* sRNA co-regulation by transcription factors and involvementin virulence**.

Multidrug resistant bacterial infections (MDR) represent major public health issues and an economic burden. In 2019, 1.2 million deaths were attributed to BMR infection (Murray et al, 2022). These bacteria are a threat because of the difficulty in finding effective treatments for infections. Among ESKAPE, *S. aureus* is an asymptomatically borne commensal bacterium in about 30% of the population. It is involved in more than half of skin infections but is also responsible for bacteremia, osteomyelitis and endocarditis. The success of an *S. aureus* infection is linked to its ability to coordinate the expression of many virulence factors at key moments, but also to adapt to environmental changes. To this end, *S. aureus* expresses protein regulators such as transcription factors (TF) and 2-component systems (TCS), as well as RNA-type regulators such as RNA regulators (sRNA). Despite a crucial role in transcriptional and post-transcriptional regulation, corresponding genes are rarely essential to the survival of the bacteria. This is because TF and sRNA are embedded in complex regulations where several FT regulate the same sRNA, and where several sRNA regulate the same target (Menard et al, 2022).

These redundancies are a determining factor explaining the absence of essentiality, as well as we expect that the joint absence of a couple or pool of regulators could lead to stronger phenotypes (Rachwalski et al, 2022). Previous work has focused on this in the study of thedirect targets of the TF SarA, in which we showed that 11 of the 37 sRNAs repressed by SarA were also repressed by another TF, CodY (Augagneur et al, 2020; Oriol et al)). Functional redundancy would mask the importance of some regulators. The objective of this thesis project is therefore to reveal the redundancies between sRNA through the identification of common regulators, using the yeast one hydrid system. Then, we will study the phenotypes related to the joint absence or overexpression of sRNA in particular in virulence, antibiotic resistance and biofilm formation. We will work with a series of ~12 sRNAs, selected based on current knowledge.

We will use the heterologous one hybrid system in the yeast *Saccharomyces cerevisiae* as a screening tool to identify the *S. aureus* TF regulating the selected *S. aureus* sRNAs. In this context, a 75 TF expression bank of *S. aureus* will be built. Similarly, the 12 strains of the carrieryeast for which the 12 regions promoting the sRNAs of interest have been cloned upstream of the reporter gene will be built. Screening will allowthe identification of all TF regulating each sRNA of interest. Next, we will select the most promising regulatory duos (sRNA or TF) in terms of functional redundancy to build double mutants in *S. aureus*. These mutants will be studied in relation to their phenotype in virulence, antibiotic resistance and biofilm formation.

For the study of *in vivo* virulence, we opted for the larval model *Galleria mellonella*, which is ethically more responsible (Ménard et al, 2021). However, this model requires the development and/or adaptation of tools. This is why, in parallel with the development of screening tools by heterologous one hybrid, it will be necessary to test and choose the tools allowing bacterial visualization andquantification (bioluminescence or fluorescence) during infection. This will be done through the integration of reporter genes into a neutral locusof the bacterial chromosome. This system built in a wild strain will be transferred by phage transduction into the mutants we study. The identification of regulatory duos essential for growth, virulence, biofilm formation or the development of antibiotic resistance could, in thelonger term, lead to the identification of antibacterial targets.

The candidate will need to have a good knowledge of molecular biology as well as microbiology. He/she will need to feel able to perform experiments on the *Galleria mellonella* larval model.

Contact: **Astrid Jouvante Rouillon** (astrid.rouillon@univ-rennes1.fr)
Phone number: 02 23 23 43 94
U1230_BRM, Rennes, France