Regulation and impact of post-transcriptional modifications of *Staphylococcus aureus* tRNAs

Synopsis/Abstract

Staphylococcus aureus is a commensal bacterium in humans, but it is also a major pathogen responsible for numerous nosocomial and community infections. Its infectious success in various tissue environments is associated with a wide range of virulence and stress response factors that enable it to develop complex invasion and immune escape strategies. The expression of virulence factors is finely regulated by various molecular mechanisms such as transcription factors, two-component systems and regulatory RNAs.

However, a new translational regulation mechanism involves post-transcriptional modifications of RNAs (Antoine et al. 2021). These modifications, produced by specific enzymes, are present on all types of RNA and can modulate translation efficiency by influencing RNA structure, codon usage bias, interaction with other molecules or the efficiency of ribosome action. In collaboration with Stefano Marzi's group (IBMC, Strasbourg), we have identified 57 modification enzymes, 37 of which affect *S. aureus* transfer RNAs. We were able to show that the genes encoding these modification enzymes are extremely well conserved within the *S. aureus* specie and are therefore part of the core genome. We therefore hypothesized that these **post-transcriptional modifications of tRNAs could play an essential role in an infectious context and finely regulate the translation of bacterial factors**.

The thesis project will be divided into three work axes:

Axis 1: Regulation of enzymes and post-transcriptional modifications of tRNAs

Although the genes encoding tRNA modification enzymes are highly conserved, the control of the expression of these genes remains to be explored. We have put forward the hypothesis that these genes could be induced in response to stress and could be essential in an infectious context.

To explore this hypothesis, several avenues of study will be considered:

- The transcriptional expression of the various modifying enzymes will be analyzed by RT-qPCR on *S. aureus* cultures subjected to various stresses related to the infectious context: antibiotic stress, oxidative stress, iron deficiency, acidic pH, in contact with blood or tissue.....
- In parallel, we will seek to decipher the regulatory network involved in the expression of these modification enzymes using the method described by Weiss et al. 2022. To do this, we will use the Nebraska library of *S. aureus* mutants (available in the laboratory) and select all mutants deficient in transcriptional regulators and two-component systems. We will analyze the expression of modification enzyme genes in these different mutants.

Axis 2: Determining the bacterial factors whose expression is altered by tRNA modifications.

To understand how tRNA modifications can influence the virulence and physiology of *S. aureus*, we will seek to identify the bacterial factors affected by these modifications. To this end, 'clean' mutants deficient in the modifying enzymes will be created. Transcriptomic (RNAseq) and proteomic analyses will be carried out and comparisons made. To identify the factors impacted at the translational level, we will look for factors that are differentially expressed in proteomics but whose transcriptional level is not altered.

Axis 3: Deciphering the mechanisms of translational regulation.

The modulation of virulence factor expression by tRNA modifications can be caused by several mechanisms, such as an improvement in the translational efficiency of codon-enriched genes whose decoding is facilitated by these modified tRNAs (Aubee et al., 2016) or a reduction in translation fidelity due to the stimulation of reading frame shifting (Fleming et al., 2022). To determine the mechanisms by which enzymes indirectly regulate translation, several approaches are planned:

- Decoding efficiency will be compared using reporter gene into which we will introduce the target codons of the modified tRNAs. The translation efficiency of wild-type and deficient strains for the modifying enzymes will be compared.
- Ribosome profiling will be carried out to quantify the translation efficiency of each mRNA and to determine the rate of translation.

- Quantification of the reading frame shift will be evaluate by constructing a pair of reporter genes under the same promoter, separated by an intergenic region enriched in the codons studied and possessing a stop codon.

All the data generated by this thesis project will enable us to **understand the role and importance of post-transcriptional modifications of *S. aureus* tRNAs during the infectious process**, and thus to **describe a new molecular mechanism for regulating virulence**.

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Proposed collaboration within ArchiFun network (not mandatory at this stage):

Proposed list of secondments (not mandatory, but recommended if known already):

Main ArchiFun theme involved:

Host-pathogen interactions.

Mechanisms of bacterial resistance.