Master's Thesis Project Proposal

Title: *Dissecting agr-Type Specific Regulation and Activation in Staphylococcus aureus Using Clinical Strain Collection and Isogenic Strain Models*

Supervisor: Annelies Zinkernagel
Co-supervisors: Julian Bär (julian.baer@usz.ch) & Mariane Pivard (mariane.pivard@usz.ch)
Location: University Hospital Zurich
Duration: 8 to 12 months **Start Date:** upon agreement, as early as possible

Candidate profile: We are looking for a highly motivated and strongly organized Master student with previous experience in wet-lab microbiology. Experience in molecular biology is beneficial but not required.
Application process: Please apply by writing an email in English with a short motivation letter and a CV to both co-supervisors.
Working environment: The lab is situated within the University Hospital and affiliated with the Department of Infectious Diseases and Hospital Epidemiology and collaborates closely with the research group of Silvio Brugger. The combined group encompass approximately 25 people consisting of Master Students, PhDs, postdocs, senior scientists, medical personnel and lab management with a diverse mix of Microbiology, Immunology, Computational Biology and Clinical Epidemiology expertise. Weekly lab meetings, multiple monthly department-wide seminars and two lab-retreats per year allow a broad scientific exchange. Non-work-related activities are organized often.

Project Background and Rationale

The accessory gene regulator (*agr*) system in *Staphylococcus aureus* is a key quorum-sensing mechanism that controls virulence factor expression. While four *agr* types (I–IV) are known, their functional differences remain poorly understood due to confounding effects of diverse genetic backgrounds in natural isolates. Preliminary data also suggest that *agr*-III strains exhibit reduced or delayed activation, despite being associated with successful clinical lineages. Understanding whether these differences are intrinsic to the *agr* types or shaped by lineage-specific factors is crucial for elucidating *agr*'s role in pathogenesis and its potential as a therapeutic target.

Objectives

1. **To determine whether *agr*-III strains universally exhibit reduced *agr* activation**, and if so, identify genetic or phenotypic correlates.
2. **To compare the regulatory strength and downstream effects of all four *agr* types** in a clean, isogenic background to assess intrinsic differences.
3. (Secondary) To explore whether the chromosomal distance between the *agr* locus and its target genes influences regulatory strength.

Methodology

Part 1:
- **Strain Collection and Typing:** Utilize large in-house clinical strain collection and collaborate with internal and external partners to obtain a diverse panel of colonizing and infecting *S. aureus* isolates with *agr*-III background. Confirm *agr* type and clonal complex (CC) via PCR and sequencing.
- **Phenotypic Assays:** Perform hemolysis assays and transform strains with *agr*-GFP-reporter plasmid and use if for *agr* activity measurements across *agr*-III strains to assess activation levels.
- **Bioinformatics:** Analyze *agr* locus sequences and correlate SNPs with phenotypic outputs (e.g., hemolysis, GFP signal).

Part 2:
- **Isogenic Strain Construction:** Generate a panel of 20 isogenic strains by replacing the native *agr* locus in four genetic backgrounds with each of the four *agr* types, including Δ*agr* controls.
- **Gene Expression Analysis:** Use RT-qPCR to quantify expression of key virulence genes at defined time points, guided by existing RNA-seq data.

Part 3:
- **Genomic Distance Analysis:** Investigate whether gene expression changes correlate with chromosomal distance from the *agr* locus.

Expected Outcomes

This project will clarify whether *agr*-type-specific differences are intrinsic or context-dependent and provide a robust isogenic model system for future *agr*-related studies. It may also uncover novel regulatory principles such as spatial effects on gene expression.

Relevance

This work contributes to a deeper understanding of *S. aureus* virulence regulation and may inform the development of *agr*-targeted antivirulence therapies. It builds on and complements ongoing work on *agr* inhibition.