Characterizing the growth patterns of novel *S. aureus* mutants; both in vitro and ex vivo

Dominic Estes, Wynter Doyle, Bryn Lien, and Dr. Madison Collins
Department of Biological and Physical Sciences, Montana State University Billings

ABSTRACT

Staphylococcus aureus (*S. aureus*) is a ubiquitous commensal of the human anterior nares that is estimated to permanently colonize ~30% of the population. *S. aureus* is also a predominant infectious pathogen that causes significant morbidity and mortality and bears a considerable burden on the healthcare industry. Options for treating this "superbug" are dwindling at an alarming rate. Although initially being considered a hospital-acquired pathogen, community-associated strains have emerged. These strains have the ability to avoid normal immune cell killing and cause disease in healthy individuals. Mechanisms for how *S. aureus* can escape the defenses of the body are incompletely defined. Previously published work has demonstrated a role for the two-component gene regulatory system, SaeR/S, in *S. aureus* and that the SaeR/S system influences the ability for the immune system to perform effectively[1–3]. Although initially considered a two-component system, SaeR/S is actually composed of four genes: *saeP*, *saeQ*, *saeR*, and *saeS* and the roles of *saeP* and *saeQ* are yet to be fully discovered. It is speculated that SaeR/S inhibits the proper function of attacking innate immune cells that circulate in the blood, although the role of the accessory proteins on the blood are completely unknown. We have begun to characterize the role of these accessory genes by using a clinically relevant strain of *S. aureus* USA300 and isogenic deletion mutants (deficient in either *saeP* and *saeQ*; USA300Δ*saeP* and USA300Δ*saeQ*, respectively). Experiments first began by quantifying the growth patterns of these mutants during in vitro broth culture, as well as, ex vivo during growth in heparinized human whole blood. These studies will help to fill clinically relevant gaps in our understanding of how *S. aureus* escapes the host immune system to advance disease during septicemic infection. Defining how this pathogen can survive immune defenses in our circulatory system can help identify new potential targets for the design of therapeutics.

INTRODUCTION

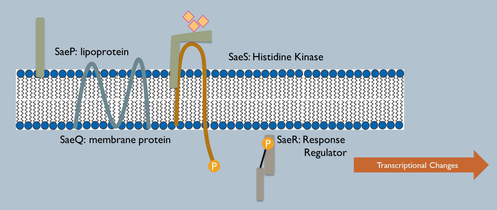
Staphylococcus aureus (S. aureus) is a commensal organism that permanently colonizes 1 in 3 people in the anterior nares.

S. aureus senses and adapts to changing environmental conditions (colonization vs disease). This "sensing" is done using two-component gene regulatory systems and mainly the SaePQRS system. It has been shown that *S. aureus* needs SaeR/S to cause disease, but very little is known about the role of the *saeP* and *saeQ* genes in the virulence of this bacterial pathogen.

METHODS

To determine in role of *saeP* and *saeQ* in *S. aureus*, isogenic mutants deficient in either gene were generated in the USA300 *S. aureus* background.

Firstly, we must determine the growth characteristics of these new mutants during in vitro culture.

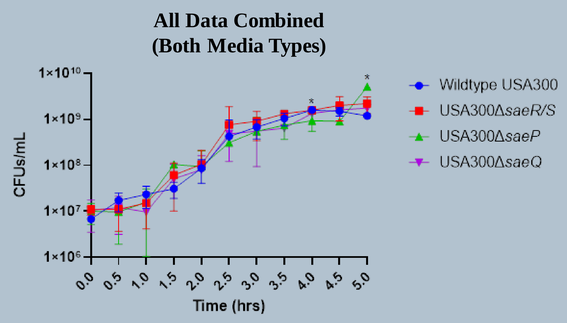


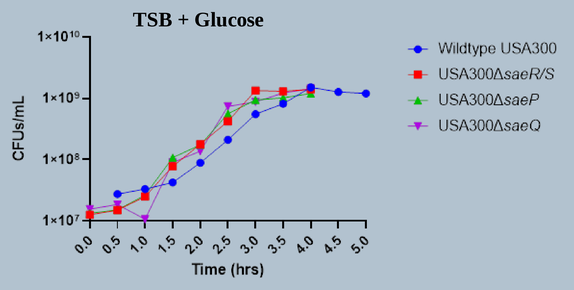
S. aureus growth dynamics- *in vitro*

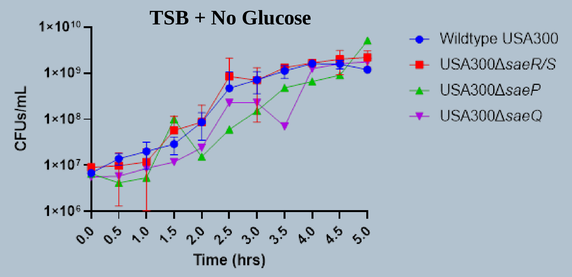
1. Add 20mLs of Tryptic Soy Broth (TSB) supplemented with or without glucose into a 125mL pyrex flask
2. Combine *S. aureus* bacteria from freezer stocks with growing media Incubate covered flasks at 37°C while shaking overnight (approximately 15 hours).
3. The following day, combine 20mls of fresh media with 200uL of overnight culture. This is a 1:100 dilution.
4. Cultures are reincubated at 37°C while shaking and timepoint samples were collected every 30 minutes
5. During timepoint collection, 100uL of culture was diluted in serial dilutions. Several dilutions on each timepoint were plated on Tryptic Soy Agar (TSA).
6. Colony forming Units (CFUs) were enumerated on TSA plates the following day and graphs were generated.

RESULTS

Preliminary results of *S. aureus* growth curves suggest little difference in CFU concentration during most timepoint collections. No significant differences were detected in the mid exponential phase of growth (hour 1.5-2.5). There was a significant different between USA300 wildtype and USA300Δ*saeP* CFUs during the 4.0 and 5.0 hour timepoints (early stationary). One-way ANOVA p-value *<0.05; n=6







When comparing which media type is more supportive of S. aureus stain growth, data suggests TSB without glucose may lead to slight increases in overall growth. Outliers exist in both data sets and more replicates will clarify growth curve trends. TSB+ Glucose, n=1; TSB+ No Glucose. n=5.

CONCLUSIONS

The research in the Collins lab aims to understand how the *SaeR/S* (or *SaePQRS*) system influences *S. aureus* virulence during human infection. Initial experiments using isogenic mutants in either *saeP* or *saeQ* suggest little difference in *S. aureus* colony forming units (CFUs) as a measure of bacterial growth. The study seeks to compare S. aureus in TSB + Glucose versus TSB + No Glucose and preliminary data demonstrates no obvious difference in media. This means further studies can be done with confidence that S. aureus strains will grow similarly with or without the presence of glucose.

FUTURE STUDIES

In the future, our lab will further our current studies of how these *S. aureus* mutants thrive during in vitro culture.

We will work to obtain more replicates of growth curves in TSB with glucose and without. Additionally, a future direction for this research will include the study of examining the difference between how *S. Aureus* grows in tryptic soy broth versus blood culture. This study will be done with each gene. To help understand if these genes impact survival in blood. It will also show how the mutants survive in heparinized human whole blood. The method of this study will be similar as will the process of data collection and measurement of growth will be done the same as in this study. These future studies will shed light on how *S. aureus* grows during human blood infection (bacteremia) and potential growth differences between in vitro and ex vivo culture can be observed.

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