Investigating gene expression related to the two-component SaeR/S regulatory system of *Staphylococcus aureus*

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ABSTRACT

Background and Objective:
The *Staphylococcus aureus* (*S. aureus)* exoprotein secretion system (SaeR/S) is a two-component protein system within *Staphylococcus aureus* that has been linked to this pathogen's ability to survive within human neutrophils (polymorphonuclear leukocytes or PMNs). Prior studies have shown that an extracellular (EC) loop, consisting of nine amino acid residues on SaeS, is vital for *S. aureus* to sense and respond to extracellular stimuli—specifically components of human PMNs.

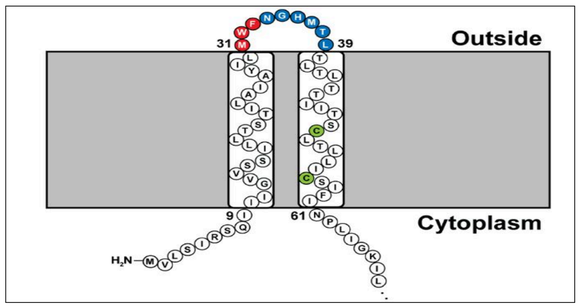


Figure 1. Shows the extracellular loop and the nine amino acid residues on SaeS; sensor protein.

Additionally, γ-hemolysin (*hlgA*) is a predominant virulence factor that targets immune and red blood cells. This toxin has been shown to be regulated by SaeR/S. New *hlgA*-GFP *S. aureus* cell strains—including point mutations of the residues on the EC loop—have been developed in order to study the role of each residue in *S. aureus* survival. All strains contained a plasmid on which the *hlgA* gene was linked with the GFP reporter. The current study sought to both characterize the activity of these strains in the presence of human PMNs as well as determine if *hlgA*-GFP fluorescence was a legitimate proxy for measuring *hlgA* expression.

Methods: We investigated the expression of *hlgA* following neutrophil phagocytosis of *S. aureus* using *hlgA*-GFP reporter strains. Spectrophotometry was used to measure GFP fluorescence within samples after being incubated for varying lengths of time.

Results: Our findings suggest that the *hlgA*-GFP reporter can be used to show *hlgA* expression at later time points (4-6 hr). However, at earlier time points (0.5-2 hr) the *hlgA*-GFP reporter was not sensitive enough to assess *hlgA* transcription. This is likely because GFP was not present in high enough quantities to be detected.

Discussion and Conclusions: The data collected in this study demonstrate that the *hlgA*-GFP reporter can be used as a proxy for *hlgA* transcription during neutrophil interaction. However, it is not sensitive enough to be used at time points earlier than four hours. Additionally, our data imply that at later time points (4-6 hr) *hlgA* may be controlled by a regulatory system within *S. aureus* other than SaeR/S. To date, there is no research outlining the regulation of *hlgA* at later time points.

METHODS

S. aureus Strains
- Wild Type: MRSA
- Complement
- Knock out Δ*saeS*
- Point Mutants →
  a) M31A
  b) W32A
  c) F33A
  d) N34A

Each point mutant has Δ*saeS* + WT *saeS* where the designated amino acid residues on the EC loop of *saeS* is mutated to an alanine.

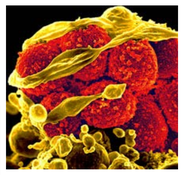


Figure 2. PMN engulfing MRSA Credit: NIAID

We used these strains to show whether or not the EC loop on SaeS is sensing specific neutrophil components. The *hlgA* gene is regulated by the SaeR/S two-component system. Our objective was to use a green fluorescent protein (GFP) as a proxy for *S. aureus hlgA* expression. The *hlgA* gene is quickly and highly upregulated in the presence of neutrophils and neutrophil components. We hypothesized that a GFP labeled *hlgA S. aureus* strain would fluoresce in response to neutrophil phagocytosis.

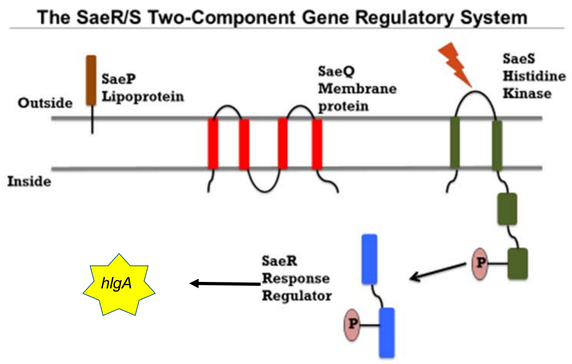


Figure 3. The SaeR/S two-component system. SaeR/S contains four components but the functions of SaeP and SaeQ are unknown. SaeS is the sensor protein which senses PMN components that turns on SaeR, the response regulator. SaeR aids in transcribing specific genes such as *hlgA.*

Growth Curves: Strains were grown overnight in tryptic soy broth (TSB). Day cultures (20 mL) were started using a 1:100 dilution of overnight culture. At every hour 1mL samples were analyzed for absorbance at 600 nm and serial diluted for colony forming unit determination (CFUs/mL). This data is important to calculate and find an accurate bacteria to PMNs ratio of 1:5. These growth curves also help in presenting the exponential growth phase and ensure that our strains do not have any growth defects.

Spectrophotometry was used to measure GFP fluorescence (excitation at 488 nm and emission 535 nm) within samples after phagocytosis by human neutrophils (PMNs) while being incubated at 37˚ celsius for up to 6 hours.

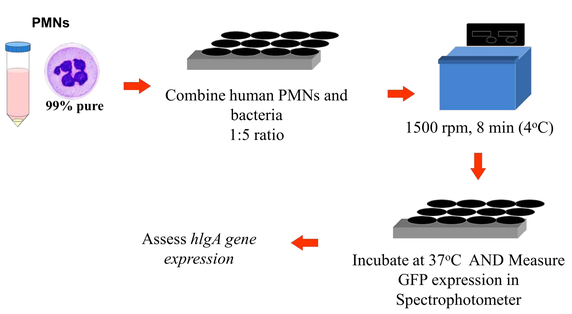
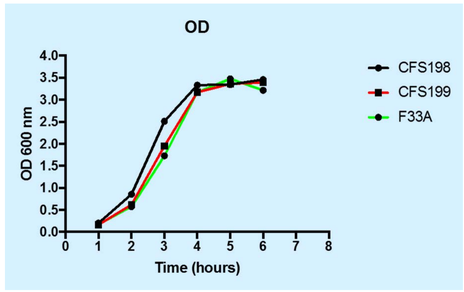


Figure 4. A schematic of the experimental setup used to measure *hlgA* expression.

GROWTH CURVE RESULTS



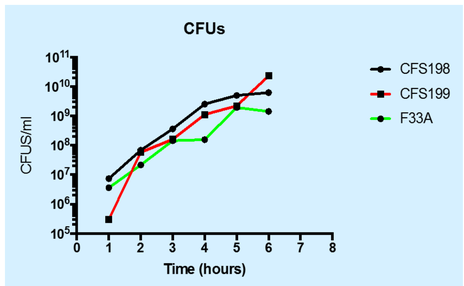
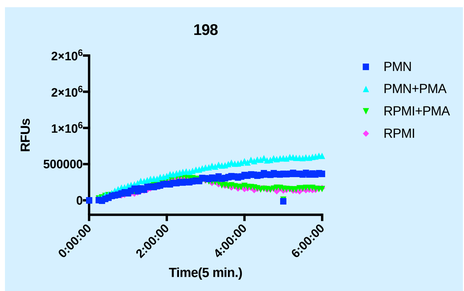


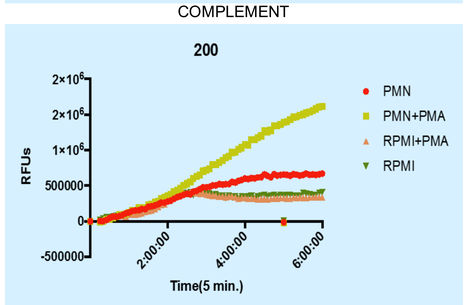
Figure 5. Growth curves of WT, knockout and point mutation (F33A) strains. The top figure shows the optical density (OD 600nm) over 6 hours. The bottom figure shows the colony forming units (CFUs) per mL at each hour.

SPECTROPHOTOMETER RESULTS

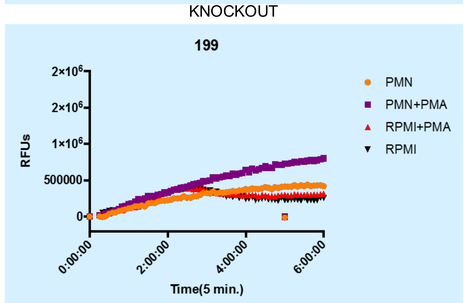
WILDTYPE



COMPLEMENT



KNOCKOUT



Figures 6: The Relative Fluorescent Units vs. Time. RFUs are taken every 5 minutes. The complement strain (200) shows a huge increase in *hlgA* expression compared to the knockout (199) and WT (198). PMA was used to activate neutrophils at faster rates, this influenced *hlgA* expression.

DISCUSSION AND CONCLUSIONS

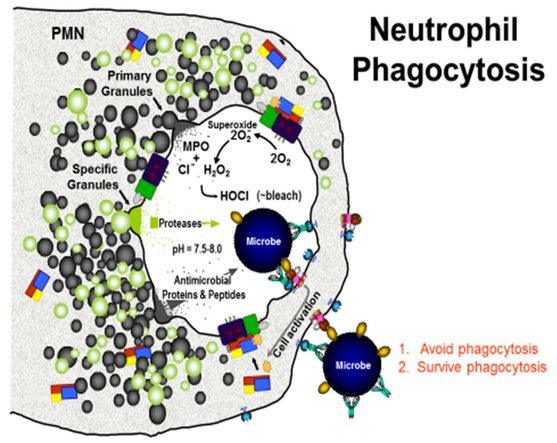
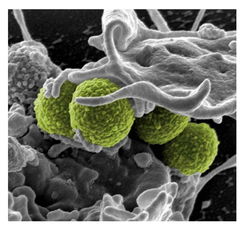


Figure 7. PMNs, are a specific type of white blood cell that defends against microbial and fungal infections. They contain granules within which contain all sorts of antimicrobial chemicals, enzymes, and reactive oxygen species that are activated from ingesting an unknown microbe. These components activate *saeS* which in turn upregulates *saeR* and target genes like *hlgA.*

- The *hlgA*-GFP reporter can be used as a proxy for *hlgA* transcription during neutrophil interaction.
- The *hlgA*-GFP reporter is not sensitive enough to be used at time points earlier than four hours.
- At later time points (4-6 hr) *hlgA* may be controlled by a regulatory system within *S. aureus* other than SaeR/S.



FUTURE WORK

We would like to investigate if other extracellular residues of SaeS are important for recognizing neutrophils and other individual components such as hydrogen peroxide. In our studies we only investigated four residues out of nine.

ACKNOWLEDGMENTS

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