The Influence of Staphylococcus Aureus Biofilm-associated Gene Mutations on Biofilm Composition

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Abstract

Staphylococcus aureus (SA) biofilms are serious impediments to immune defenses and antibiotics, making them a major factor in SA infections. Such infections can be highly lethal despite current treatments, presenting a major challenge to the healthcare industry. Previous genetic screenings of SA have revealed several genes that may be associated with biofilm formation. While many of these genes have been studied, little research has been done to examine how mutations of said genes impact biofilm composition. [1] As several therapeutic options for treating mature SA biofilms require an understanding of the biofilm's composition, an effort to better comprehend how the genes influence that composition is critical to improving current treatments and developing new ones.

In this project, we are conducting a study the biofilm phenotypes of the Je2 strain of SA with mutations in common biofilm-associated genes. By comparing the biofilm mass and composition of the wild type Je2 strain to strains containing mutated biofilm-associated genes, we hope to uncover the impact of each mutation on the composition of the biofilm matrix. To make comparisons between mutant biofilms and wild type biofilms, variants of a crystal violet assay are used to determine and characterize biofilm composition

Introduction

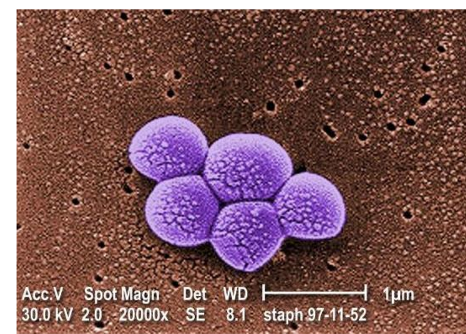


Image from http://www.cdc.gov

Figure 1: A colorized scanning electron micrograph (SEM) of methicillin resistant SA.

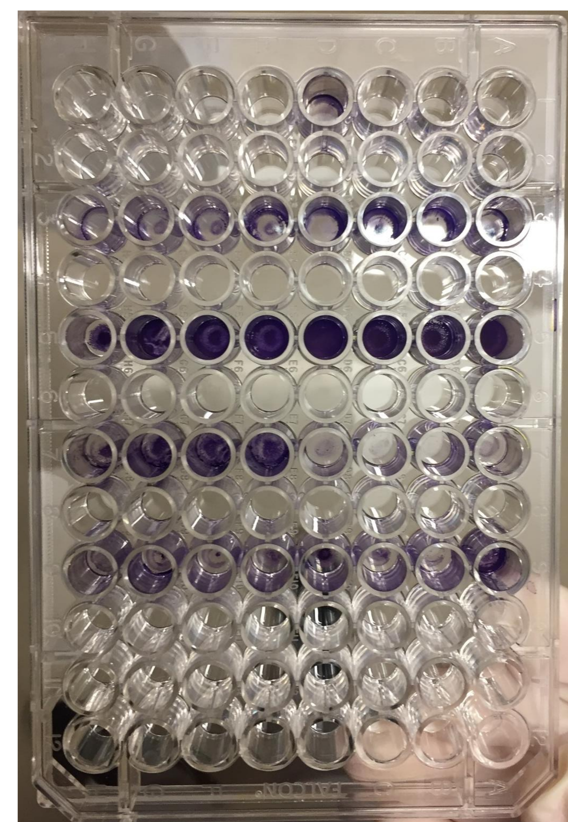
Staphylococcus aureus (SA) infection has become a considerable threat in medical settings as a main route for serious nosocomial infections. Over the past decade, the number of hospital-acquired infections has declined only slowly, despite expensive prevention efforts. Of particular concern is the rise of Methicillin-resistant SA (MRSA), known to form biofilms, which causes almost 20,000 deaths per year in the United States alone.[2]

Biofilm formation by SA is a key protection for the bacteria against antibiotics and other antimicrobials. Biofilms form when planktonic bacteria attach themselves to a surface and build an extracellular polymeric matrix composed of eDNA, protein, and sometimes polysaccharides. This polymeric shielding often creates strong antibiotic resistance, making biofilm dispersal critical to both treatment and prevention of infection. Biofilm studies are still in their infancy, and a greater understanding of biofilm structure will be critical to further therapeutic developments. [3]

Crystal Violet Assay

To calculate the average difference in biofilm production and composition between two different strains, we modified a crystal violet assay protocol performed previously by our lab. [4]

- Samples were grown up overnight in Tryptic Soy Broth (TSB) and then diluted 1:200 in a 66% TSB solution with .5% glucose.
- 200mL of each sample was added to four different wells on 96-well culture-tissue plates. The plates were then incubated at 37° C for 18 hours.
- The liquid was then dumped, leaving only the biofilms. In the experiments requiring it, DNase or Proteinase K were then added for 1 hour and dumped out.
- The biofilms were then fixed with ethanol, dyed, and washed. After drying, the crystal violet was eluted and an absorbance reading was taken at 595 nm.



JE2 Strain Mutants

Several biofilm-associated genes were selected from the results of genetic screenings performed by other labs. For each gene, we used a mutant version of the MRSA Je2 strain obtained from the University of Nebraska medical center Je2 transposon mutant library. Each mutant contained a transposon located in the target gene, inactivating the functions of that gene. The genes were selected based on the availability of their corresponding transposon mutants. A comprehensive list of the selected transposon mutants tested and the function of their mutated genes are found below: [5, 6]

Gene	Function
fnbA/fnbB	Bind fibronectin, a glycoprotein of the extracellular matrix.
clfA/clfB	Bind fibrinogen, an extracellular precursor to fibrin, which creates rigid and fibrous meshworks.
ebpS	An integral membrane protein that binds elastin, a component of the extracellular matrix that provides elasticity and resiliency.
purH	An enzyme catalyzing the last step in purine synthesis in the pentose phosphate pathway. Through an unknown mechanism, this contributes to biofilm growth and infection persistence.
coa	Binds fibrinogen and aids in fibrin mesh formation.
sarA	Prevents the repression of PNAG, a glucosamine critical to biofilm formation.
icaA	Key to formation of polysaccharide intercellular adhesin (PIA), an essential component of polysaccharide-containing biofilms.
agrA	Regulates the production of the regulatory RNAIII molecule. The ultimate impact of this regulation on biofilms is still under investigation.
acnA	Aconitate hydratase, an enzyme of the tricarboxylic acid cycle
alt	An autolysin that regulates growth and cell lysis
Controls	lacA, lacB, and malABC mutants have disabled genes that do not have any association with biofilms. Since we grow up the bacteria in glucose, lactose and maltose will not be utilized.

DNase and Proteinase K Treatments

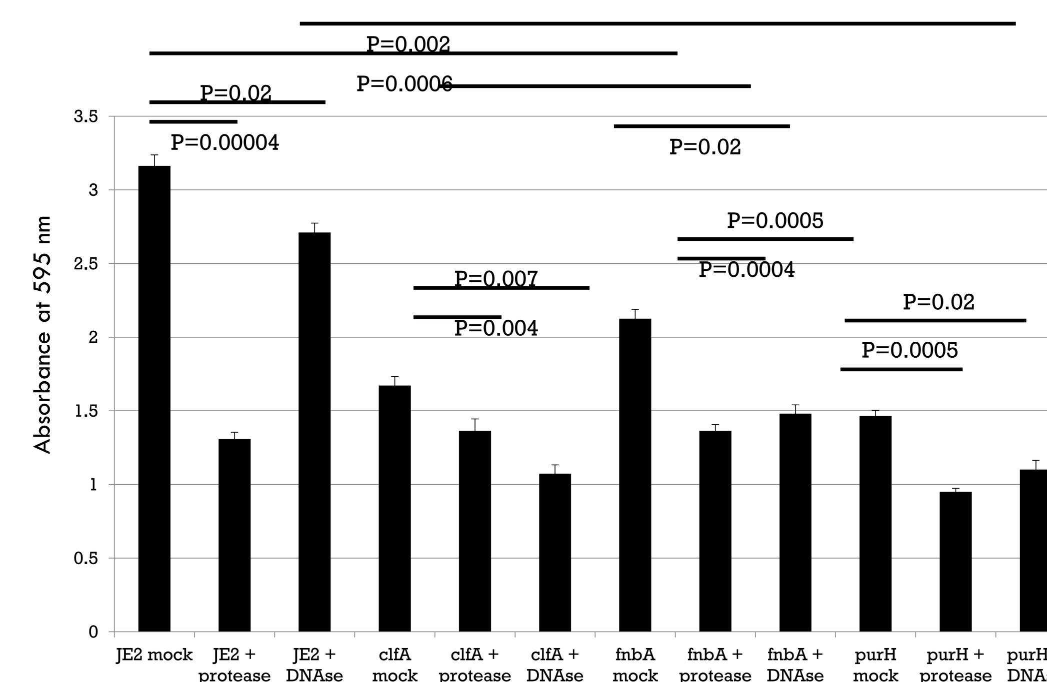


Figure 3: The overall masses of the biofilms measured after strains were subjected to DNase, Proteinase K, or mock treatments. Strains that had demonstrated a significant reduction in overall biofilm production compared to wild type were chosen for these treatments.

These early DNase and Proteinase K treatments already show an interesting pattern. The Proteinase K treatments of the wild type, clfA, and fnbA samples cause similar reductions in mass, suggesting that knocking out clfA or fnbA reduces the protein content of the biofilm.

Wild Type and Mutant Avg. Biofilm Masses

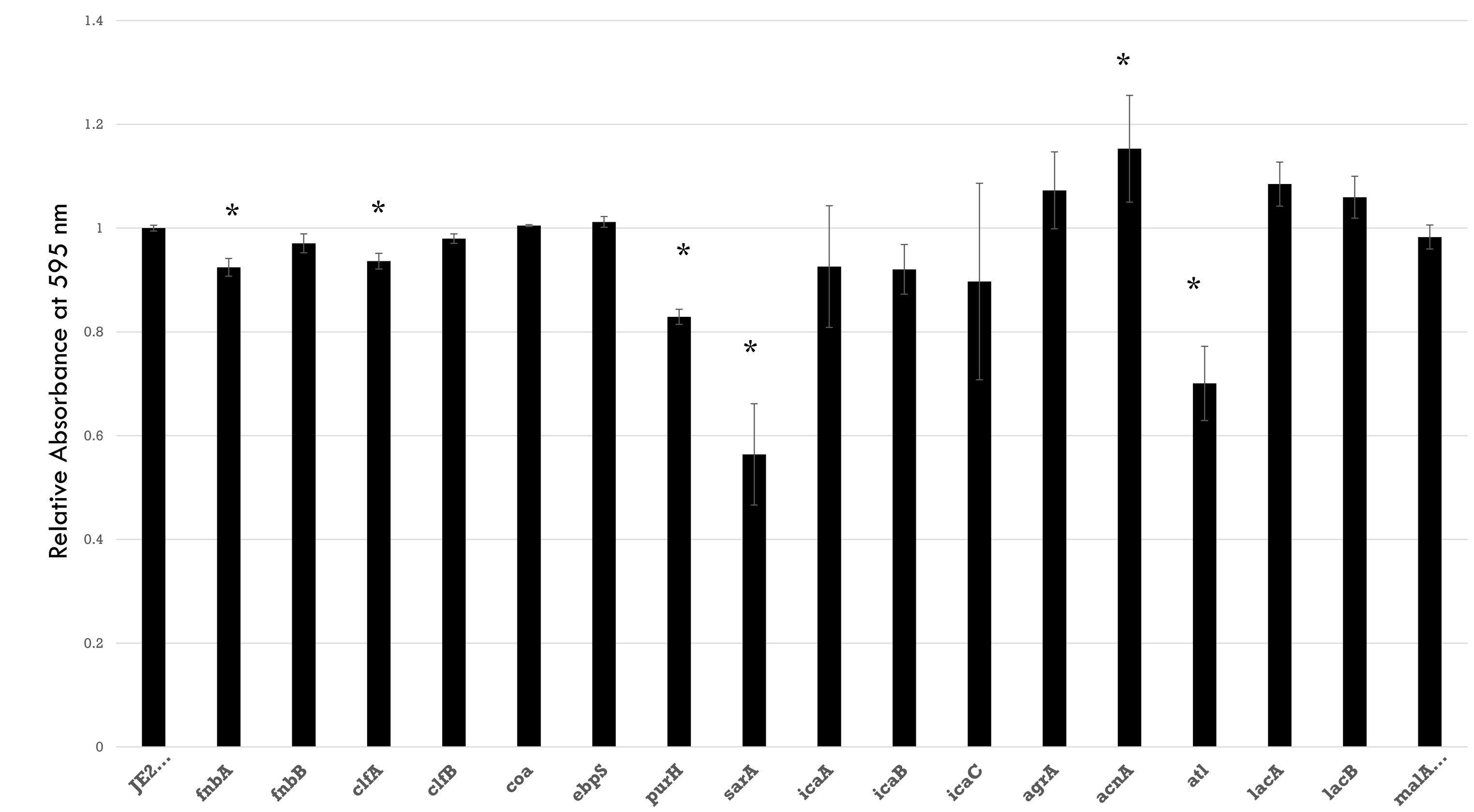


Figure 4: A comparison of the average biofilm mass of each of the Je2 mutants and the Je2 wild type. This diagram represents all of the current data normalized, with the Je2 wild type mass given a value of 1. The stars indicate those mutants which were significantly different from the wild type strain.

A preliminary comparison of the average biofilm masses produced by Je2 wild type and selected mutants showed that some mutations caused a statistically significant reduction in biofilm mass. The purH, sarA, and atl mutations caused a reduction of about 20% or more. Interestingly, acnA caused an *increase* in the mass of the biofilms. This data correlates with the work of other labs which have shown that an acnA mutation will upregulate sarA expression.[7] Our data indicates that sarA is an important gene in regulating overall biofilm mass, and as such, it would make sense that increasing sarA expression would increase the overall mass of the biofilm.

Conclusion

Thus far, we have determined that mutations in the fnbA, clfA, purH, sarA, and atl genes produce significant differences in biofilm mass when compared to wild type Je2. Interestingly, acnA knockouts actually result in comparatively higher biofilm masses. Furthermore, based on data from the Proteinase K treatments, we suspect that mutations in the clfA and the fnbA genes primarily cause a reduction in a biofilm's protein content.

Our future research will focus on continuing to perform Proteinase K and DNase treatments on the other mutant strains that displayed a statistically significant difference in their overall biofilm mass when compared to wild type JE2.

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