

Role of *sarA* and *agr* in Insulin Modulation of *Staphylococcus aureus* Biofilm Formation

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Abstract

The focus of this study is to determine whether insulin, quorum signaling and biofilm formation are under regulation of *agr* and *sarA* regulation. Deletion strains *agr*⁻, *sarA*⁻, and *agr-sarA*⁻ as well as their parent strain 8325-4 were tested with and without glucose (0.1% and 0.2%) and/or physiologic levels of insulin (2μU/mL, 20μU/mL, and 200μU/mL). In the absence of *sarA* and *agr*, there was a marked suppression of biofilm levels in the presence of insulin, indicating that insulin modulation of biofilm formation appears to be regulated, in part, by *sarA* and *agr*.

Keywords: Glucose; Quorum Compound; Signaling Compound; Hormone

1. Introduction

The ability of *S. aureus* to form biofilms is essential in its establishment and maintenance of infectious processes, e.g., foot ulcers in individuals with type 2 diabetes. *S. aureus* biofilm formation during infection in individuals with uncontrolled type 2 diabetes occurs in the presence of insulin and glucose. Bacterial infections, including surgical site infections (SSIs), are a common and serious complication of diabetes. *Staphylococcus aureus* is a major cause of SSI in diabetic patients [1,2]. However, the role of insulin and glucose in diabetes predisposing to staphylococcal infection is not fully elucidated.

Biofilm formation and stability are dependent on the environment. Promotion, or inhibition, of biofilm formation occurs in response to available nutrients, including pathway metabolites, quorum-signaling compounds, and the genetic profile of the organism [3]. Temporal expression of many of the virulence determinants, including biofilm in *S. aureus*, is under the control of several genetic loci, including *agr* and *sarA* [4-6]. The accessory gene regulator (*agr*) is a critical system that controls population density-associated gene expression (quorum-sensing). At low cell densities, low-*agr* activity is associated with biofilm formation activity. When the quorum population density is reached, the *agr* system is activated, decreasing cell-surface colonization factors. The *Staphylococcus* accessory regulator A (SarA) system is a global regulator that promotes biofilm formation, such as the *icaRA* and *bap* operons, adhesion, and toxin production. SarA modulation of gene expression depends on environmental conditions. SarA enhances bacterial colonization, particularly in device-related and chronic infections. Conversely, in *sarA* mutants, the increased protease production can disrupt biofilms. SarA also activates the *agr* quorum sensing system to coordinate gene expression. SarA's role in biofilm formation is epistatic to *agr*. When *agr* is highly expressed, SarA's repression of proteases is the overriding factor that promotes biofilm formation. For example, in MRSA strains, such as USA300, which have high *agr* expression, *sarA* is required for robust biofilm development.

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2. Materials and Methods

Glucose was dissolved and diluted in Luria Broth (LB) for use. Insulin (Humulin) was diluted in LB for use. Overnight cultures were inoculated into homologous LB to a final concentration of 10^5 CFU/ml, then added to various concentrations of glucose and/or insulin in honey-comb plates (200 μ L/well). The plates were incubated for 24 hrs (37 $^{\circ}$ C, shaking). Controls consisted of organisms grown in LB alone. After incubation, the plates were washed, dried, and stained with crystal violet (300 μ L). Bound stain was dissolved in absolute alcohol (300 μ L) with absorbance determined (580nm). Controls consisted of organisms grown in LB alone. Experiments were done in quintuplets and repeated once. Data were evaluated by analysis of variance (ANOVA; GraphPad InStat 3.06 for Windows, GraphPad Software Inc.). Mean values were considered significantly different at $p < 0.05$ (*).

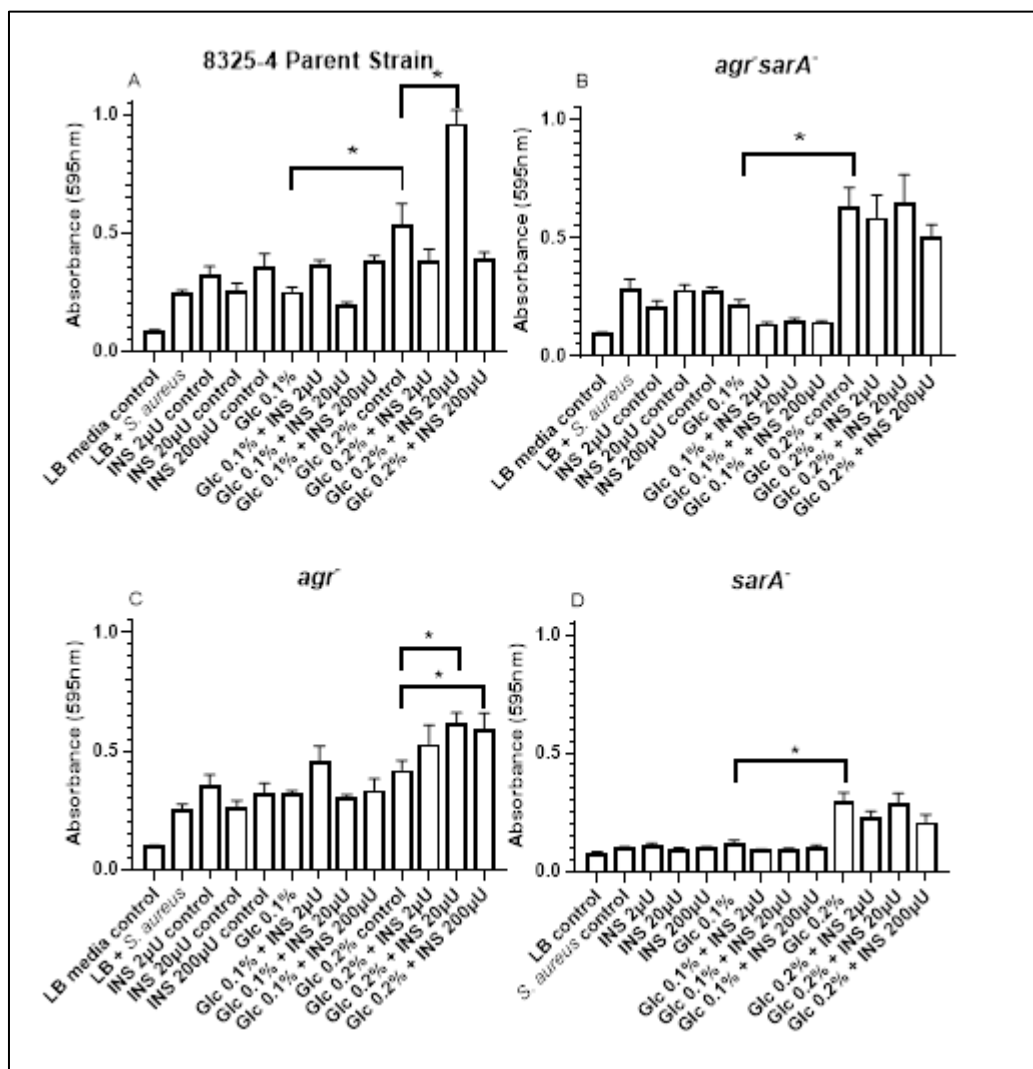


Figure 1 Effect of insulin and glucose on biofilm formation by *S. aureus*. A. Parent strain 8325-4 biofilm formation in response to insulin and glucose. B. *S. aureus agr-sarA*- biofilm formation in response to insulin and glucose. C. *S. aureus agr*- biofilm formation in response to insulin and glucose. D. *S. aureus sarA*- biofilm formation in response to insulin and glucose. * SEM $p \leq 0.05$ as compared to homologous control

3. Results and Discussion

Biofilm formation in *S. aureus* is regulated, in part, by *sarA* and *agr* via the extracellular signal of Agr, a post-translationally modified peptide containing a thiolactone [7] (Figure 1 A-D). Previous studies have shown that insulin is an inter-kingdom quorum signaling molecule that, together with glucose, modulates biofilm formation [8-11]. This response of *S. aureus* is glucose and insulin-concentration specific [12]. Whether the insulin effect is also under the regulation of *sarA* in epistatic coordination with *agr* has not been determined. For the parent strain, 8325-4, the

presence of insulin alone did not significantly affect biofilm formation, as has been reported previously for quality control and clinical *S. aureus* isolates (Figure 1A) [12]. However, the addition of glucose 0.2% significantly increased biofilm production as compared to medium & *Staphylococcus* alone or glucose 0.1%. When glucose 0.2% and insulin 20μU/mL are given together, a synergistic positive effect on biofilm formation is measured. In contrast, insulin did not affect the double negative *S. aureus agr-sarA*⁻ strain. Without *agr* and *sarA* (Figure 1B), *S. aureus* still responded to glucose in a concentration-specific manner with regard to its biofilm formation. Glucose at 0.2% resulted in significantly ($p \leq 0.05$) increased biofilm production. In contrast, the presence of *sarA* and absence of *agr*⁻ (Figure 1C) restored the response to glucose 0.2% and insulin (20μU/mL or 200μU/mL) to that similar to the parent strain, but not to the addition of glucose 0.2% alone. Interestingly, the presence of *agr* together with the deletion of *sarA* (Figure 1D) resulted in a pattern of biofilm formation that, while significantly less (4-6-fold) than that measured for the double negative strain *agr-sarA*⁻, the pattern of biofilm formation in response to glucose was similar. Glucose 0.2% significantly increased biofilm production as compared to medium & staphylococcus at 0.1% glucose. Insulin, regardless of concentration, had no effect.

4. Conclusion

The findings from this study indicate that *sarA*, in a glucose concentration-specific manner, plays a role in the regulation of *S. aureus* response to insulin modulation of biofilm formation. Furthermore, although *agr* is reported to be important in *S. aureus* response to endogenous quorum signaling compounds, it appears to be independent of insulin-mediated biofilm formation signaling response in *S. aureus* to glucose. In addition, biofilm formation appears to be regulated, in part, by *sarA* and *agr*. Taken together, these findings indicate that insulin is an inter-kingdom quorum signaling compound in *S. aureus* that plays a role in modulating biofilm formation, partially under the control of *sarA*.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

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