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Determining the effects of the Type Seven Secretion System in *Staphylococcus aureus* innate immune interactions

Introduction

Staphylococcus aureus is the most common pathogen causing skin and soft tissue infections in the United States[1] and is demonstrating increased infection rates despite antibiotic usage. Two routes are predominant for the spread of *S. aureus* infections in developed nations[2]. Healthcare-associated infections are increasing as the incidence of methicillin-resistant *S. aureus* (HA-MRSA) rises. Further, hypervirulent community-acquired methicillin-resistant *S. aureus* (CA-MRSA) strains have led to an increase in infections in otherwise healthy populations.

CA-MRSA is more virulent than HA-MRSA due to a number of genetic factors that allow it to evade the immune system[3]. Another virulence factor important for immune evasion is the highly conserved Type Seven Secretion System (T7SS) is a virulence factor that appears not just in CA-MRSA but across many gram-positive pathogens.

The T7SS is an understudied yet influential virulence secretion system present within *S. aureus*. Previous studies show the T7SS to be important for establishing murine abscesses and recurring infections[4]. Further, the T7SS secretes proteins responsible for evading the immune system[5,6]. However, the role the T7SS plays in interactions with the innate immune system remains poorly understood.

As such, the primary focus of my research is to elucidate the difference in *S. aureus* infections when a functional T7SS is present and when it is removed. My research intends to further identify the mechanisms in which the T7SS interacts with components of the innate immune system.

Methods

To establish if there was differential virulence of *S. aureus* with and without the T7SS in an innate immune environment, I adapted the *Drosophila melanogaster* septic infection model from Hobbs *et al.*[7]. The USA300 LAC-JE2 bacterial strain was used as the CA-MRSA with a functional T7SS. A transposon knockout of the *essC* gene in LAC-JE2 (*essC::NΣ*) functionally disables the T7SS by preventing EssC, the main ATPase of the T7SS, from functioning. *D. melanogaster* were infected with a tungsten needle after light etherization via a pinprick in the thorax. The flies were allowed one hour to recover before counting surviving flies for the starting population. Survival curves were constructed by counting survivors once a day (n=50).

Following the *D. melanogaster* survival analysis, I hypothesized that the differential survival of LAC-JE2 and *essC::NΣ* in the model was due to a difference in interactions with macrophages[5]. To confirm this, I tested for the survival of LAC-JE2 and *essC::NΣ* within the macrophage environment using RAW 264.7 murine macrophages. I infected macrophages with a multiplicity of infection of 25 bacteria per macrophage, as it was enough to ensure bacterial survival for 72 hours but not so much to overburden the macrophages.

To determine potential causes for a difference in survival between LAC-JE2 and *essC::NΣ* within a macrophage, I created an assay designed to identify interactions between the T7SS and a primary mechanism the macrophage uses to kill engulfed bacteria. This was a reactive oxygen species (ROS) assay. ROS is created by the macrophage via the production of superoxides, which dismutes to H_2O_2. Thus, I used a variety of dilutions of H_2O_2 added to cultures of LAC-JE2 and *essC::NΣ* to determine any differential survival to ROS.

Results

The *D. melanogaster* assay showed the expected results with a significant difference ($p \leq 0.05$) in survival between LAC-JE2 and *essC::NΣ*.

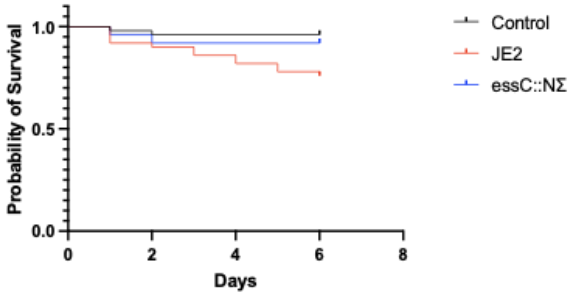

Figure 1: Kaplan-Meier survival curve shows a steady decline of LAC-JE2 infected flies while *essC::NΣ* survival plateaus. $p \leq 0.05$ at six days.

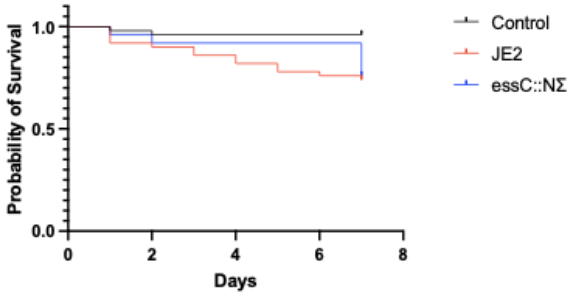

Figure 2: Kaplan-Meier survival curve shows a drop in survival for *essC::NΣ* on day seven due to a crack in the media that killed the flies.

However, the experiment will need to be repeated due to an unexpected crack forming in the media and killing *essC::NΣ* infected flies at day 7, preventing the full 14-day trial. Following the promising results of the *D. melanogaster* assay, I moved on to the macrophage assay as I waited to be able to repeat the flies.

Considering the promising results of the fly assay, I wanted to determine how the T7SS interacts with individual components of the innate immune system. I have found a ~1 log difference in survival between LAC-JE2 and *essC::NΣ*, with LAC-JE2 being able to survive better within the macrophage. Timepoints were taken for 72 hours with a concentration of points in the first 6 hours, where the largest variations in survival are expected. LAC-JE2 and *essC::NΣ* had similar killing in the first 24 hours, though LAC-JE2 shows a clear survival advantage at 72 hours.

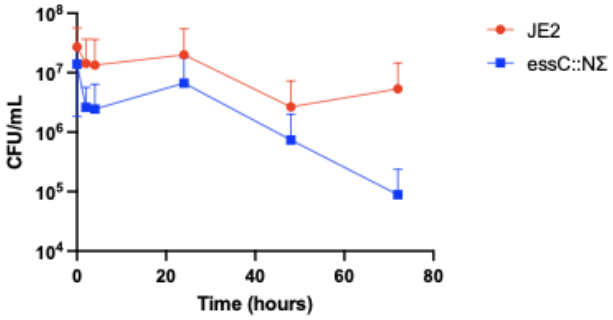

Figure 3: Survival of LAC-JE2 and *essC::NΣ* within RAW 264.7 macrophages in CFU/ml. LAC-JE2 had a growing survival advantage in later time points.

To determine if LAC-JE2's survival advantage within macrophages was due to increased tolerance to ROS produced by the macrophages, I performed a ROS tolerance assay. Preliminary results indicate no

significant difference in ROS survival between LAC-JE2 and *essC::NΣ*, and as such that survival differences within the macrophage are not due to differential ROS tolerance.

Conclusions and Future Work

While *S. aureus* remains a highly prevalent pathogen, the mechanisms of virulence within the T7SS remain poorly understood. The work from this study has shown that the T7SS plays a key role in the interactions between *S. aureus* and the innate immune system. Further work within this project will continue to elucidate the mechanisms of these interactions through the exploration of the role the T7SS plays in antimicrobial peptide tolerance and the modulation of ROS production within macrophages. Future work will also include *S. lugdunensis*, a closely related yet poorly characterized species. Thoroughly understanding the interactions of the T7SS with the innate immune system will be crucial for the future development of treatment strategies for *S. aureus.*

References:

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