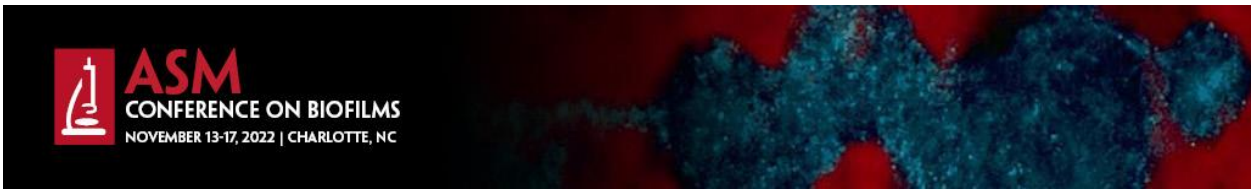

Mechanisms of protein-dependent Staphylococcal biofilm formation

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Staphylococcus aureus is one of the leading causes of healthcare-associated infections, in part because of its propensity to aggregate and form biofilms on catheters and other indwelling devices. These aggregates and biofilms are more resistant to antibiotics, making treatment difficult and often requiring removal of the device. *S. aureus* can aggregate using multiple protein-dependent mechanisms, either directly through surface protein repeat domains that dimerize and bring cells together or alternatively through bridging mechanisms with host matrix proteins. For the repeat domain interactions, the large surface protein SasG is one of the main players responsible for biofilm formation. SasG is composed of two domains, an N-terminal ligand binding domain called the A domain and a C-terminal repetitive stalk called the B domain that is sortase-anchored to the cell wall. The B domain repeats can dimerize to form twisted cable-like structures, which join neighboring cells together and contribute to biofilm accumulation. There is evidence that the A domain must first be removed before the B domains can interact, although the protease(s) responsible for processing SasG are not known. We have found that proteases present in human saliva are capable of cleaving SasG at a specific site within the A domain. In addition, processing by these host proteases leads to aggregation in strains that express SasG at high levels. For the alternative biofilm mechanism, *S. aureus* can bind dimeric host proteins like fibrinogen as a bridge to bring cells together in a process called clumping. Surface proteins clumping factor A (ClfA) and B (ClfB) are the main sortase-anchored proteins responsible for this pathway. We have determined that *S. aureus* can control clumping through the activity of the ArlRS two-component regulatory system, and its downstream effector MgrA. Inactivation of any part of this cascade leads to removal of MgrA repressive functions and upregulation of other giant surface proteins like Ebh and SraP. We discovered these giant proteins interfere with the ability of ClfA/B to interact with host matrix proteins. We hypothesize that either of these aggregation mechanisms provides a means for *S. aureus* to protect itself in the presence of host defenses and antimicrobials. We have identified inhibitors that interrupt these pathways as a novel approach for the pharmacological treatment of *S. aureus* biofilm infections.