ABSTRACT
PROBIOTIC COMPOUNDS INHIBIT STAPHYLOCOCCUS AUREUS BIOFILM FORMATION, REDUCE VIRULENCE, & IMPROVE ANTIBIOTIC SENSITIVITY

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Multidrug-resistant (MDR) bacterial infections are a top five global public health threat, causing 2.8 million infections and 35,000 deaths annually in the US alone. *Staphylococcus aureus* is one of the most clinically important MDR pathogens in the world with infections leading to high rates of morbidity and mortality in both humans and animals. This bacterium’s ability to form protective biofilms further complicates classical antibiotic interventions, highlighting the need for new therapeutics with novel mechanisms of action. The goal of this dissertation was to investigate antibiofilm mechanisms employed by probiotic bacteria to reduce *S. aureus* virulence and mitigate antimicrobial resistance evolution in this pathogen.

After isolating and screening 1123 *Bacillus* isolates, I investigate the antibiofilm mechanisms deployed by *Bacillus subtilis* 6D1, an agriculturally sourced strain capable of inhibiting *S. aureus* biofilm growth and disassembling mature biofilm. I demonstrate this activity is driven predominantly through Agr quorum sensing interference (QSI) and can be attributed to the production of multiple surfactin isoforms and a novel compound that, together, are more potent than commercially obtained HPLC grade surfactin. Furthermore, this mixture of compounds reduced *S. aureus* virulence in human intestinal cell lines via stimulation of adaptive immune responses. I next report on the phenotypic and genotypic adaptations incurred by *S. aureus* after long-term exposure to *B. subtilis* 6D1 cell-free extracts (CFEs) that possess the antibiofilm mechanisms described above. Phenotypic screening of *S. aureus* lineages revealed those evolved in the presence of CFEs were less competitive in a biofilm, less virulent, and did not develop resistance to a variety of antibiotics with different mechanisms of action. Additionally, *B. subtilis* 6D1 CFEs maintained antibiofilm activity against all *S. aureus* lineages regardless of evolutionary condition and were protected from developing mutations in both competence and drug resistance pathways.

Collectively, this dissertation demonstrates the utility of probiotic mediated QSI mechanisms to maintain anti-virulence activity against *S. aureus* over evolutionary time. As global antibiotic resistant infection rates climb, exploring antipathogen mechanisms that reduce biofilm formation and improve antibiotic efficacy is essential to understand how probiotics can be leveraged to combat MDR pathogens and reduce the spread of antimicrobial resistance.