CodY-mediated Biofilm Formation in *Staphylococcus aureus*  

K. D. Mlynek¹, L. L. Bulock², M. R. Sadykov², K. W. Bayles², S. R. Brinsmade¹; ¹Georgetown University, Washington, DC, ²University of Nebraska Medical Center, Omaha, NE.

The global transcriptional regulator CodY integrates nutrient availability into the regulation of nearly all virulence genes in *Staphylococcus aureus*, including those required for biofilm development. Antithetical phenotypes of both biofilm deficiency and accumulation have previously been associated with a codY null mutation; thus, the role of CodY protein in biofilm development has remained unclear. Herein we report that codY mutants of the majority surveyed *S. aureus* clinical isolates form dense cell aggregates (clumps) during planktonic growth and develop a viscous biofilm under biologically relevant flow conditions. Biochemical studies indicate that both biofilm development and cell clumping are dependent on a self-produced extracellular DNA (eDNA) matrix, suggesting eDNA release and/or adherence to the matrix is required for the observed phenotypes. Experiments utilizing mixed populations of fluorescently labeled strains revealed that wild-type cells failed to co-localize in clumps with the respective isogenic codY mutant. Co-culturing the non-clumping LAC codY mutant with a clumping SA564 codY mutant did not impede cell clumping. Surprisingly, the LAC codY mutant was excluded from the eDNA aggregates, suggesting the absence of clumping is likely rooted in a cell associated trait and not caused by secreted factors. Fortuitously, we isolated a spontaneous suppressor mutant with additional lesions in icaB (polysaccharide intracellular adhesin synthesis) and in mraY (peptidoglycan synthesis) that failed to aggregate. Co-cultures of the suppressor mutant and the codY strain resulted in mixed aggregates, suggesting a defect in eDNA release. Taken together, our results suggest that codY mutant biofilms arise in part from alterations to the cell envelope that lead to cell lysis and subsequent eDNA release, as well as a yet-to-be identified factor produced in the codY mutant that mediates the cell-eDNA matrix interaction.