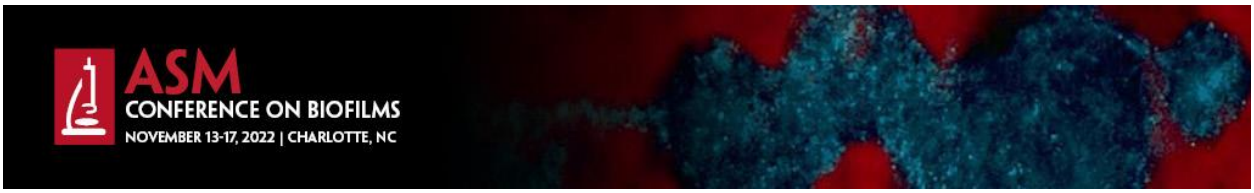

Staphylococcal Secreted Cytotoxins are "Competition Sensing" Signals for *Pseudomonas aeruginosa*

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Interspecies interactions between two notorious opportunistic pathogens that dominate chronic infection in the cystic fibrosis (CF) airway, *Pseudomonas aeruginosa* and *Staphylococcus aureus,* critically affect CF disease progression and treatment outcome. Here, we used a combination of microscopy, genetics and chemo-selective proteomics approaches to uncover a previously unknown "competition sensing" model where *Staphylococcal* secreted cytotoxic phenol-soluble modulin (PSM) peptides are key interspecies signals that mediate *P. aeruginosa* antagonistic response.

First, we discovered that *P. aeruginosa* is attracted to *S. aureus* and travels up a gradient of *Staphylococcal* secreted factors using type IV pilus mediated motility. Attraction results in *P. aeruginosa* invasion into *S. aureus* microcolonies, dispersal of *S. aureus* cells from the edge of the colonies, and enhanced susceptibility to *P. aeruginosa* antimicrobials. Accordingly, a PSM-deficient *S. aureus* exhibited increased survival in co-culture compared to wild-type.

To further understand how *P. aeruginosa* responds to *S. aureus* cytotoxins, time-resolved proteomic analysis of *P. aeruginosa* immediate global response to PSM peptides and *S. aureus* cell was performed. This further revealed systematic induction of the pyoverdine biosynthesis cluster and the type VI secretion system (T6SS)—an interspecies weaponry that loads and fires effectors targeting both mammalian and prokaryotic cells. Single-cell microscopy of a fluorescent reporter of T6SS activity confirmed that PSMs are sufficient to induce *P. aeruginosa* deployment of T6SS. Finally, *P. aeruginosa* deficient in T6S were less competitive with *S. aureus.* Thus, we propose a model whereby *P. aeruginosa* senses *S. aureus* secreted peptides from a distance, pre-empts an attack by upregulating pyoverdine and T6SS and attacks using a wolfpack-like strategy surrounding, invading, and disrupting *S. aureus* colonies. Invasion and disruption aids in local concentration of secreted antimicrobials and potentially increases contact-mediated mechanisms of competition. These observations provide a paradigm-shifting model for T6SS-mediated antagonism by a Gram-positive bacterium and its secreted signal.