Comparing the response of *Pseudomonas aeruginosa* to natural, synthetic, and semi-synthetic antimicrobials

Opportunistic pathogens encounter antimicrobials during infection as well as outside the host. At sub-inhibitory levels, antimicrobials induce subtle changes in expression of stress-related and metabolic genes, which has led to the idea that antimicrobials not only function to inhibit bacterial growth but also act as cues. However, little is known about how bacteria respond to natural versus synthetic or semi-synthetic antimicrobials. The Gram-negative opportunistic pathogen *Pseudomonas aeruginosa* causes various acute and chronic infections in immunocompromised individuals and accounts for 15% of all nosocomial infections. *P. aeruginosa* is both intrinsically resistant and can acquire resistance to multiple antimicrobial classes. Here, we employed two genome-wide sequencing methods to: 1) elucidate the transcriptional response to sub-inhibitory levels of 17 natural, synthetic, and semi-synthetic antimicrobials; and 2) identify genes required for resistance to these antimicrobials. We hypothesized that *P. aeruginosa* has evolved an adaptive response to antibiotics commonly produced by soil microbes whereas synthetic or semi-synthetic antimicrobials will not elicit an adaptive response. Using RNA-sequencing, we found that the transcriptional response varied from having as little as 0 genes to as many as 500 genes differentially expressed upon exposure to sub-MIC levels of an antimicrobial. In general, *P. aeruginosa* had a more robust transcriptional response to natural antimicrobials than semi-synthetic antimicrobials. A closer look at the genes identified by RNA-seq indicated that oxidative stress-related genes are induced upon exposure to several different classes of antimicrobials. To identify genes required for resistance to these antimicrobials, we profiled the fitness of ~300,000 *P. aeruginosa* transposon mutants during growth in the presence of sub-inhibitory levels of antimicrobials using Transposon-sequencing (Tn-Seq). Tn-seq revealed that oxidative stress-related genes are also critical fitness determinants for multiple classes of antimicrobials. Our results reveal that *P. aeruginosa* possesses unique responses to natural, synthetic, and semi-synthetic antimicrobials and we anticipate this work will continue to yield important insights in the involvement of antimicrobials in growth inhibition as well as communication.