Using high-throughput technologies to understand mechanisms of predation

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Project Goals: ENIGMA -Ecosystems and Networks Integrated with Genes and Molecular Assemblies use a systems biology approach to understand the interaction between microbial communities and the ecosystems that they inhabit. To link genetic, ecological, and environmental factors to the structure and function of microbial communities, ENIGMA integrates and develops laboratory, field, and computational methods.

Abstract:

Microbial communities are highly dynamic impacted by diverse biotic and abiotic factors in an ecosystem. One of the key biotic factors that impact the community dynamics is the antagonistic behavior by the members of the community. Like bacterial viruses/phages, bacterial predators that kill and feed on other nearby prey bacteria account for large percentage of bacterial mortality and are widespread in diverse ecosystems. Similarly, phage tail-like bacteriocins (PTLBs) are highly potent, variable spectrum bactericidal agents known to impact microbial community dynamics. Though predator-prey interactions have been studied over deacdes, our molecular understanding of how prey-recognition and specificity of interaction works is still unclear[1]. In particular, there is a scarcity of such information on bacterial predators (phages and bacterial predators) and bactericidal agents in heavy metal contaminated soil and water bodies such as Oak Ridge FRC field site. For example, the metagenomic sequence data from Oak Ridge FRC site samples shows the presence of bacterial predators such as *Bdellovibrio* and *Bacteriovorax* species, however their role in structuring the community observed is less clear. The discovery of predator-prey interaction specificity determinants and predator resistance mechanisms would open new avenues for the dissection of ecosystem function.

We identified PTLB biosynthetic gene clusters in eleven of a set of twelve closely related Oak Ridge *Pseudomonas* isolates[2]. We focus on five killing interactions, and two resistant interactions, between PTLBs purified from producing strains, and genome-wide random bar code transposon-site sequencing (RB-TnSeq) mutant libraries[3] of target strains. PTLB production was confirmed and characterized by transmission electron microscopy and proteomics analyses. To survey host factors involved in PTLB mediated target killing, we performed pooled fitness assays with the RB-TnSeq libraries, using PTLBs as stressors. Initial analysis of our genome-wide fitness data suggests that specific lipopolysaccharide residues–likely serving as binding receptors–are involved in killing by different PTLBs. We aim to determine the atomic structure of the tailocin tail fibers in complex with the LPS in order to understand the recognition mechanism used by Tailocins to bind to and kill bacteria.

We have successfully enriched bacterial predator *Bdellovibrio* from the serially enriched water sample from Oak Ridge FRC field site. We are currently refining our methods to isolate clonal isolate of predators that grow on different target hosts. Our next steps will include characterization of their host-range and prey cognition determinants using genome-wide libraries of target isolates from the same environment.

References

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