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7th Annual SEA-PHAGES Symposium Abstract

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Exploring the Impact of Bacterial Growth on Mycobacteriophage Protein Expression by Mass Spectrometry

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The fundamental knowledge of phage has led to many applications in biotechnology. Phages are engineered to be nano-carriers for drugs and anti-bacterial agents based upon their target specificity and simple virion structure. The limited information of phage-host interactions and expression of phage proteins, however, constrains maximizing their full potential for biotechnology.  
  
The life cycle of a phage begins with attachment to the host cell. After injecting genomic DNA into a bacterium, a phage may further enter either lytic or lysogenic cycle. In lytic cycle, the phage replicates DNA and produces virions by hijacking host machinery, eventually resulting in lysis and the release of virions. The lysogenic cycle is distinct from lytic cycle, as phage DNA is integrated into the bacterial chromosome and replicated during bacterial cell division. Under certain conditions of a lysogenic cycle, the phage DNA can be released from the host chromosome and subsequently enter a lytic cell cycle.   
  
Previously, we reported a novel mass spectrometry (MS) method that facilitated the identification of peptides produced in phage-infected Mycobacterium smegmatis (M. smegmatis) culture. We conducted further analysis by searching mass spectra in six-reading frames of the phage genomes as reported by Pope et al, 2014, and detected many unexpected out-of-frame peptides that were not predicted by the genome annotation (manuscript in preparation). We hypothesize that the peptides we observed were produced in response to specific phases in the growth cycle of the host and highlight the importance of examining the phage-host system in more detail. In order to test this hypothesis, we are currently investigating the impact of bacterial cell cultures from different growth phases (exponential, stationary and death) on phage life cycle and protein expression. We selected phage with and without canonical integrase proteins to infect M. smegmatis and harvest at different phases of growth for subsequent protein extraction and analysis by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).  
  
Our long-term goal is to identify those proteins that are mediating the lytic and lysogenic cycle and characterize peptide markers that are expressed during key transition points in the life cycle of the phage. Our findings will provide a better understanding of the interactions of the phage-host system over time and contribute towards novel applications in biotechnology.