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# QUANITIFYING AMINOARABINOSE ON BACTERIAL CELL MEMBRANES

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*Yersinia pestis* is the bacterium responsible for plague, which has decimated human populations throughout history, with the Black Death being the most infamous instance. Plague remains a modern-day concern since hundreds of infections occur every year and, although generally treatable, *Y. pestis* strains have been identified in Madagascar that are resistant to multiple antibiotics. Additionally, because it causes a highly contagious disease with a high mortality rate if left untreated, *Y. pestis* has been used as a biological weapon by humans for centuries, and it is currently classified by the CDC as a Tier 1 Select Agent.

*Yersinia pestis* is usually transmitted to humans via flea bite, causing bubonic plague. To undergo transmission to mammals, *Y. pestis* must first be able to initiate and maintain a successful infection in the flea. It was recently confirmed that *Y. pestis* modifies lipid A in its outer membrane with aminoarabinose (Ara4N) and that this modification confers resistance to cationic antimicrobial peptides (CAMPs) and allows the bacteria to successfully infect fleas. In the absence of Ara4N, CAMPs can form pores in the outer membrane, leading to cell death. The current method of detecting Ara4N modifications in *Y. pestis* is by mass spectrometry using isolated lipid A, which requires expensive instruments and has its own constraints regarding quantifiable data. To better investigate the mechanisms of CAMP resistance in *Y. pestis*, a new method to detect and quantify the amount of Ara4N-modified lipid A is needed. This project used phage display to search for peptide sequences that would specifically bind Ara4N-modified lipid A that could be modified to use as a tool for Ara4N detection and quantification.

Phage display uses bacteriophages that display different short peptides on their outer surfaces to study specific interactions with target molecules. This project used two commercial phage display kits (NEB Ph.D. 7-mer and 12-mer libraries) with whole cell *Y. pestis* strains as targets. Phage libraries were first bound to a mutant *Y. pestis* strain that cannot synthesize Ara4N to remove general membrane-binding phages. Remaining unbound phages were then used against wild type (WT) cells that do produce Ara4N-modified lipid A. Bound phage were eluted from the WT cells and then amplified via *E. coli*. This binding and amplification process, called panning, was repeated twice more per library, and then individual phages were tested for their binding abilities against mutant and WT *Y. pestis* and sequenced.

After several rounds of panning with the 7-mer library, it was observed that recovered phages bound to both the mutant and WT *Y. pestis* cells, indicating the recovered sequences did not bind Ara4N specifically. The 12-mer library has undergone three rounds of panning and assays to determine the binding specificity of the resulting sequences are in process. If peptide sequences that specifically bind Ara4N-modified lipid A are not identified from the 12-mer library, other avenues will be pursued, such as using isolated LPS as the target for phage display or using aptamers in a similar manner. If an Ara4N-binding molecule can be identified via these methods, it would greatly enhance future research on mechanisms of CAMP resistance in *Y. pestis*.