Identification of *Yersinia pestis* Genes Regulated by the PhoP-PhoQ Sensory System in the Flea Vector

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Yersinia pestis, the etiological agent of plague, cycles between two dissimilar hosts: the mammalian host and the flea vector. Effective vector transmission of *Y. pestis* by the flea vector requires formation of a bacterial biofilm within the flea foregut. Recently, the two-component sensory system PhoP-PhoQ was found to be critical for the development of a biofilm within the flea vector. To characterize the role of the PhoPQ system *in vivo*, global expression profile was determined between the *Y. pestis* KIM6+ Δ*phoP* and the corresponding wildtype strain using microarray analysis. To identify a gene expression set unique to the flea gut, the experiment was designed with the following four conditions: 1) LB-MOPS planktonic culture at 23°C into exponential phase, 2) LB-MOPS planktonic culture at 23°C into stationary phase, 3) *in vitro* flowcell biofilm, and 4) *in vivo* infected flea (*Xenopsylla cheopis*) midguts. For each condition, cells were enzymatically lysed and total RNA isolated using an RNeasy mini-column (Qiagen). Any contaminating DNA was removed through DNase treatment, and lack of residual DNA contamination was verified by RT-PCR. The quality and quantity of the extracted RNA was assessed by UV spectrophotometry and the Agilent Bioanalyzer 2100, respectively. Total RNA was then amplified to generate biotin - labeled aRNA using the MessageAmp II-Bacterial RNA Amplification Kit from Applied Biosystems. The incorporation of biotinylated nucleotides was confirmed by a streptavidin gel-shift assay. A standardized quantity of fragmented aRNA was hybridized to a custom Affymmetrix *Y. pestis* array chip. A statistical analysis revealed 161 genes to be differentially expressed in the *Y. pestis* KIM6+Δ*phoP* relative to the wild-type in the flea midgut;133 genes were downregulated and 28 genes were upregulated. In addition, 10 differentially expressed genes appeared to have a potential PhoP binding motif. Further examination of the expression profile is required to identify PhoP regulated phenomena important for biofilm formation and survival and infectivity of *Y. pestis* in the flea vector environment.