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Regulation of *Yersina pestis* Virulence by AI-2 Mediated Quorum Sensing

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Motivation

The proposed research was motivated by an interest in understanding *Y. pestis* virulence mechanisms and bacteria cell-cell communication. It is expected that a greater understanding of virulence mechanisms will ultimately lead to biothreat countermeasures and novel therapeutics. *Y. pestis* is the etiological agent of plague, the most devastating disease in human history. *Y. pestis* infection has a high mortality rate and a short incubation before mortality.[1] There is no widely available and effective vaccine for *Y. pestis* and multi-drug resistant strains are emerging. *Y. pestis* is a recognized biothreat agent based on the wide distribution of the bacteria in research laboratories around the world and on the knowledge that methods exist to produce and aerosolize large amounts of bacteria.[2]

We hypothesized that cell-cell communication via signaling molecules, or quorum sensing, by *Y. pestis* is important for the regulation of virulence factor gene expression during host invasion, though a causative link had never been established. Quorum sensing is a mode of intercellular communication which enables orchestration of gene expression for many bacteria as a function of population density and available evidence suggests there may be a link between quorum sensing and regulation of *Y. pesits* virulence. Several pathogenic bacteria have been shown to regulate expression of virulence factor genes, including genes encoding type III secretion, via quorum sensing.[3-5] The *Y. pestis* genome encodes several cell-cell signaling pathways and the interaction of at least three of these are thought to be involved in one or more modes of host invasion.[2] Furthermore, *Y. pestis* gene expression array studies carried out at LLNL have established a correlation between expression of known virulence factors and genes involved in processing of the AI-2 quorum sensing signal. [6]

Scope

This was a basic research project that was intended to provide new insights into bacterial intercellular communication and how it is used to regulate virulence in *Y. pestis*. It is known that many bacteria use intercellular signaling molecules to orchestrate gene expression and cellular function. A fair amount is known about production and uptake of signaling molecules, but very little is known about how intercellular signaling regulates other pathways.[7] Although several studies demonstrate that intercellular signaling plays a role in regulating virulence in other pathogens, the link between signaling and regulation of virulence has not been established. Very little work had been done directly with *Y. pestis* intercellular signaling apart from the work carried out at LLNL. The research we proposed was intended to both establish a causative link between AI-2 intercellular signaling and regulation of virulence in *Y. pestis* and elucidate the fate of the AI-2 signaling molecule after it is taken up and processed by *Y. pestis*. Elucidating the fate of AI-2 was expected to lead directly to the understanding of how AI-2 signal processing regulates other pathways as well as provide new insights in this direction.

Research plan and expected results

Background

Bacteria are able to communicate via chemical signals called autoinducers. Bacteria cells respond to autoinducer levels in a process called quorum sensing.[7] Quorum sensing is used to sense population density changes and to orchestrate cellular function, including regulation of virulence in some pathogens, as a community (figure 1). A fair amount of work has been done elucidating pathways for the production and uptake of autoinducer molecules, but little is known about how sensing of autoinducers regulates other pathways.[7] Efforts at LLNL and elsewhere

have identified a number of the autoinducer 1 (AI-1) class of molecules produced by *Y. pestis*, but very little is know about the function of the AI-2 system in *Y. pestis*.[8] The *Y. pestis* genome encodes the lsr operon (see figure 1) and we previously demonstrated that *Y. pestis* produces and takes up AI-2 activity. We also previously determined the x-ray crystal structure of lsrG from *Y. pestis*.

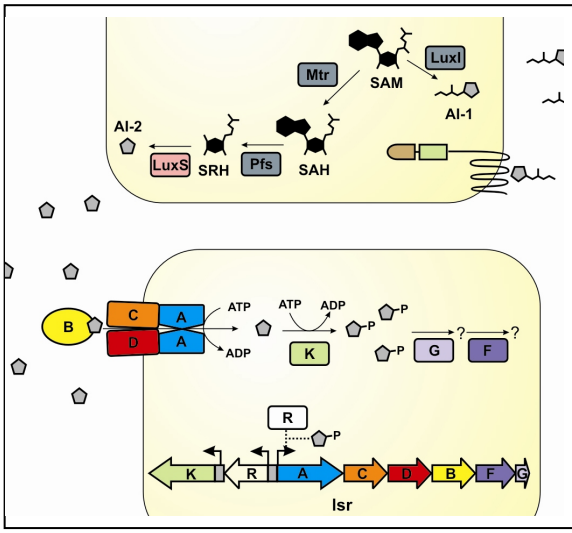

Figure 1. Bacteria quorum sensing via autoinducer molecules AI-1 and AI-2. As part of cellular utilization and metabolism of SAM, both AI-1 and AI-2 molecules are produced and secreted. Genes in the bacterial genome, in a region called the lsr operon, encode proteins responsible for uptake and processing of AI-2. lsrB binds AI-2 outside the cytosol and delivers it to the transporter made up of lsrA,C, and D. Upon uptake, AI-2 is phosphorylated by lsrK. Phosphorylated AI-2 antagonizes the transcriptional repressor, lsrR, thereby up-regulating expression of the lsr genes. lsrG and lsrF further process phospho-AI-2. Phospho-AI-2 and products of lsrF and G probably signal other pathways as well.

We originally proposed the following specific aims

We proposed to establish a causative link between the uptake and processing of AI-2 and its regulation of virulence factor genes in *Y. pestis* and to elucidate AI-2 processing pathways. We proposed to do so by pursuing three complementary lines of investigation. First, proposed to observe the effects of specific engineered genetic mutation, which alter different aspects of AI-2 uptake and processing, on expression of known virulence factor genes. Second, we proposed to track the fate of AI-2 as it is taken up and processed by *Y. petsis* using radiocarbon labeled AI-2 and to identify AI-2 metabolites by mass spectroscopy and accelerator mass spectroscopy. This is a novel approach to studying cell-cell signaling that leverages unique LLNL capabilities. Using AMS in this way is obviously generalizable, and likely a very powerful way to examine a pathogens interactions with its environment. Finally, proposed to elucidate the substrates and products of the enzymes encoded in the lsr operon, which encodes the genes for AI-2 uptake and processing (figure 1). We proposed to carry out the following tasks:

1. Engineer genetic mutant strains, or knockouts, of Y. pestis deficient in individual genes required for AI-2 production, uptake, and processing (luxS, lsrA, lsrK, lsrF, and lsrG)
2. Monitor the impact of specific knockouts on expression of known virulence factor genes (yersinia outer proteins, yops, and low calcium response genes) by RT-PCR
3. Synthesize AI-2 and radiocarbon labeling AI-2
4. Isolate radio carbon label containing metabolites generated in the cytosol of *Y. pestis* following uptake of exogenous radiocarbon labeled AI-2
5. Identify AI-2 metabolites by mass spectroscopy
6. Isolate gene products (proteins) known to be involved in AI-2 uptake and processing
7. Identify substrates and products (metabolites) in the known AI-2 processing pathway

Engineering genetic knockouts in Y. pestis KIM D27:

We attempted to generate knockout mutants using the Datsenko and Wanner one-step inactivation method of chromosomal bacterial genes using PCR products.[9] This method works by homologous recombination where a recombinase and an engineered linear

segment of DNA carrying recombinase sites are introduced into the bacteria to be genetically modified. The DNA segment is designed such that the recombinase replaces the target gene with the engineered DNA segment. We have experience using this method to knock out genes in *Y. pestis*. Regulatory restriction on this work turned out to be a much more significant hurdle than we had anticipated. Methods we use confer transient antibiotic resistance to the target organism is now highly restricsted for virulent organisms such as *Y. pestis*. Despite using avirulent strain of *Y. pestis* (KIM D27) our IBC committee required that we work under the restrictions applied to fully virulent *Y. pestis*. It took us approximately 2 years to overcome the restrictions and develop a genetic system that works in the avirulent strains of *Y. pestis* we used. We were able to generate one genetic knockout at the end of the project and have yet to test the phenotype of the mutant.

Monitoring expression of virulence factor genes
Since we were not able to produce the genetic knockouts until the end of the project we were not able to carry out this part of the proposed work.

Chemical synthesis of AI-2
We completed the chemical synthesis of AI-2 and [^{14}C]AI-2.

Tracking AI-2 metabolites with AMS
Delays in generating genetic knockouts significantly delayed the start of these experiments. We are now poised to initiate these experiments and we are pursuing support to continue this work.

Isolating proteins involved in AI-2 uptake and processing
We were able to isolate and characterize lsrB, lsrK, lsrF, lsrG and some independently folding subdomains of ypo0405. We were not able to isolate an active form of lsrR and we did not attempt to isolate lsrA, B, and C, since these are membrane associated proteins.

Elucidating metabolites of AI-2 processing
We were able to generate products of AI-2 processing, but have yet to fully characterize them.

Additional work
In addition to the originally proposed work we collaborated with computational biology to predict protein-protein interactions amongst the proteins encoded in the lsr operon and worked to experimentally validate the predicted interactions. We also obtained the crystal structure of lsrF and have crystals of AI-2 bound to lsrB.

References:

1. Zhou D, Han Y, Yang R, (2006), Molecular and physiological insights into plague transmission, virulence and etiology, *Microbes and Infection* 8: 273–284
2. NIAID Biodefense Research Agenda for CDC Category A Agents, U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, National Institutes of Health, National Institute of Allergy and Infectious Diseases 2002.
3. Laasik E, Andresen L, Mae A, (2006), Type II quorum sensing regulates virulence in Erwinia carotovora ssp. carotovora, *FEMS Miocrobiology Letters* 258: 227-34
4. Yahr1 TL, Wolfgang MC, (2006), Transcriptional regulation of the *Pseudomonas aeruginosa* type III secretion system, Molecular Microbiology 62: 631-40
5. Krin E, Chakroun N, Turlin E, Givaudan A, Gaboriau F, Bonne I, Rousselle J-C, Frangeul, Lacroix C, Hullo M-F, Marisa L, Danchin A, Derzelle S, (2006), Pleiotropic Role of Quorum Sensing Autoinducer 2 in *Photorhabdus luminescens*, Applied and Environmental Microbiology October 2006: 6439-51
6. Motin VL, Georgescu AM, Fitch JP, Gu PP, Nelson DO, Mabery SL, Garnham JB, Sokhansanj BA, Ott LL, Coleman MA, Elliott JM, Kegelmeyer LM, Wyrobek AJ, Slezak TR, Brubaker RR, Garcia E, (2004), Temporal global changes in gene expression during temperature transition in Yersinia pestis, *Journal of Bacteriology* 186: 6298-305
7. Camilli A, Bassler BL, (2006), Bacterial small-molecule signaling pathways, *Science* 311: 1113-6
8. Chen Z, Li B, Zhang J, Qin L, Zhou D, Han Y, Du Z, Guo Z, Song Y, Yang R , (2006), Quorum sensing affects virulence-associated proteins F1, LcrV, KatY and pH6 etc. of Yersinia pestis as revealed by protein microarray-based antibody profiling, *Microbes and Infection* 8: 2501-8
9. Datsenko KA, Wanner BL, (2000), One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products, *The Proceedings of the National Academy of Science*. USA 97:6640-5
10. Semmelhack MF, Campagna SR, Federle MJ, Bassler BL, (2005), An expeditious synthesis of DPD and boron binding studies, *Organic Letters* 7: 569-72
11. De Keersmaecker SC, Varszegi C, van Boxel N, Habel LW, Metzger K, Daniels R, Marchal K, De Vos D, Vanderleyden J, (2005), Chemical synthesis of (S)-4,5-dihydroxy-2,3-pentanedione, a bacterial signal molecule precursor, and validation of its activity in Salmonella typhimurium, *The Journal of Biological Chemistry* 280: 19563-8