Understanding the virulence effects of *Yersinia pestis* through the deletion of T3SS proteins

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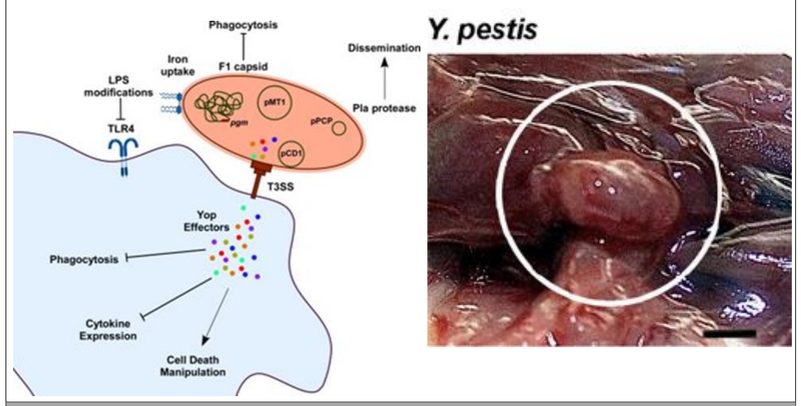
Abstract

Bubonic plague is a highly virulent disease that has caused the death of millions of humans over several centuries. It is caused by a pathogenic gram-negative bacterium called *Yersinia pestis*. *Y. pestis* contains several different virulence factors that aid in its ability to avoid the immune system and effectively kill host cells through Type three secretion system (T3SS) of *Yersinia* outer proteins (yop). YopK and YopJ are known to contribute to host cell apoptosis, while LcrQ regulates the T3SS. YopK helps fine tune the T3 secretion process while YopJ interferes with multiple signaling pathways involved with cell survival. However, whether deletion of these various proteins affects the ability of Y. pestis to kill the cell is yet to be determined. The overall objective is to understand the inter-related roles of YopJ, YopK, and LcrQ on host cell death and virulence. The objective of this work is to generate mutants that will allow us to study those interactions. We will use KIMD27-derived strains electroporated with YopJ, YopK, and/or LcrQ deletion plasmids (suicide vectors). PCR will then be performed to detect for the gene deletion or reversion to wild-type. At the end we expect there to be Y. pestis mutants with single, double, and triple deletion of YopJ, YopK, and LcrQ that can then be utilized in further research. The deletion of these T3SS virulence factors should cause reduced virulence of *Y. pestis* in vivo. This in turn will provide further insight into the pathogenesis of the Bubonic plague and help guide future research for targeted treatment of the disease as well as the development of effective detection methods.

Background

- BACKGROUND
- *Yersinia pestis* is a gram-negative pathogenic extracellular bacterium
- *Y. pestis* is known for causing bubonic and pneumonic plague.
- *Y. pestis* uses a Type 3 secretion system (T3SS) to inject host cells with virulence factors
- Virulent factors: Overall contributes to host cell apoptosis and immunity evasion
- YopJ: interferes with cell signaling pathway
- YopK: helps fine tune T3SS
- T3SS gene: LcrQ: regulates T3SS



OBJECTIVE: CREATE *Y. pestis MUTANTS WITH VARIOUS VIRULANT FACTORS DELETIONS*

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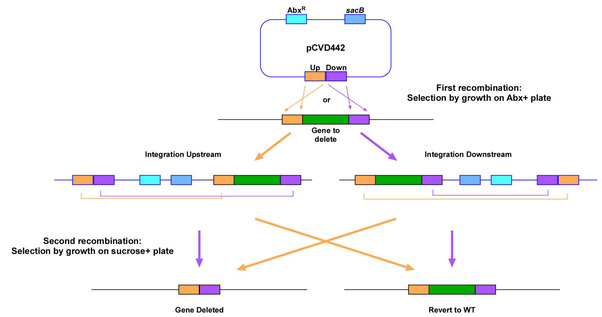
Methods

- Design primers for upstream and downstream sequences for yopJ
- Once pCVD442 has the upstream and downstream ORF of yopJ then is inserted into E. coli for replication.

- The plasmid is inserted into strand KIMD27 through electrophoresis.
- Then plated on kanamycin (KAN) positive heart infused agar (HIA) plates for positive selection. Those selected are cultured in Heart infused broth (HIB).
- Once cultured then the bacteria are diluted down and plated on sucrose plates for negative selection of the plasmid.
- Single colony units are patched on both HIA and HIA + sucrose + KAN.
- Colonies that grew ONLY on HIA plates selected for PCR.
- PCR preformed to check for yopJ deletion and control gene to ensure the strain recombination deleted the gene and did not convert back to wild type



Future Goals

- Utilize *Y. pestis* mutants in ongoing project.
- Test various mutants in immune cells (macrophages and neutrophils) to obvious virulence effects
- Understand the inter-related roles of YopJ, YopK, and LcrQ on host cell death and virulence

Results

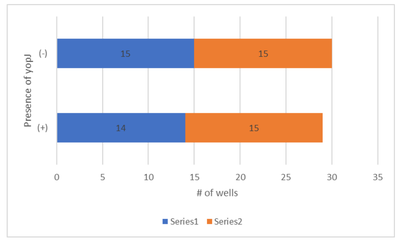


Figure 1. Recombination results of *Y. pestis* . Series 1 was the first batch, conversion to ΔJ was 51.7%.

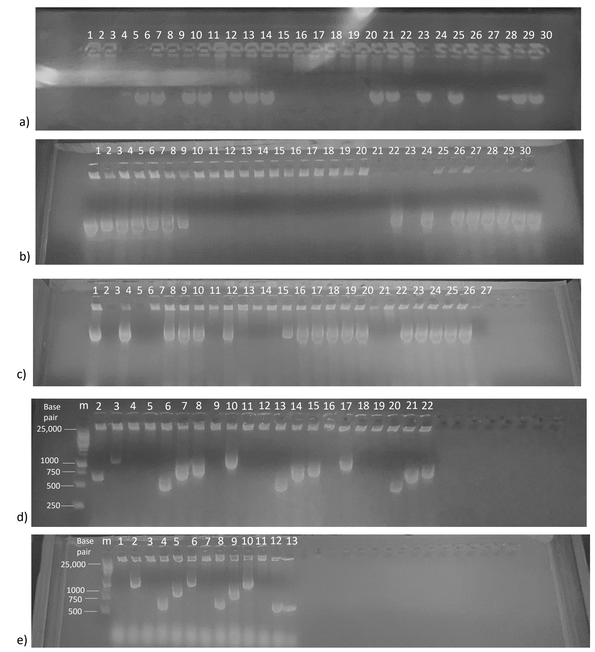


Figure 2. *Y. pestis* mutant dection. a) Mutant screen of ΔQ strains for presence of yopJ, 29 wells **b)** Mutant screen of ΔKQ strains for presence of yopJ **c)** Mutants screen for the presence of yopH, 17 confirmed yopH presence **d)** Confirmation PCR screening for yopJ, yopH, LcrQ, yopK, CafI, Pla, and ArrT for three strains of ΔJKQ **e)** Confirmation PCR screening for yopJ, yopH, LcrQ, and yopK for three strains of ΔJQ

References

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