

In vitro Analysis of Differential Expression of the *Yersinia pestis* F1 capsule and V antigen

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Outline

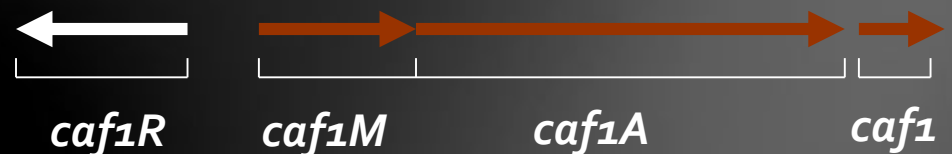
- Background
- Research Objectives
- Methods
- Results
- Conclusions
- Applications
- Acknowledgements

Background

- *Yersinia pestis*
 - Gram-negative rod in family Enterobacteriaceae
 - Bubonic and Pneumonic Plague
 - Bubonic – 50% mortality
 - Pneumonic – 100% mortality
 - Flea bite or aerosol contact
 - Varying growth conditions

Background

- Outer Membrane Proteins
 - Virulence factors
 - Environmentally regulated
 - F1 capsule antigen
 - Forms a large gel-like capsule on the cell surface
 - Anti-phagocytic properties
 - Encoded from a temperature-regulated operon
 - Strains lacking F1 capsule are still virulent
 - V antigen (Lcr)
 - Transports virulence proteins out of the cell
 - Modulates host immunological response
 - Required for full virulence



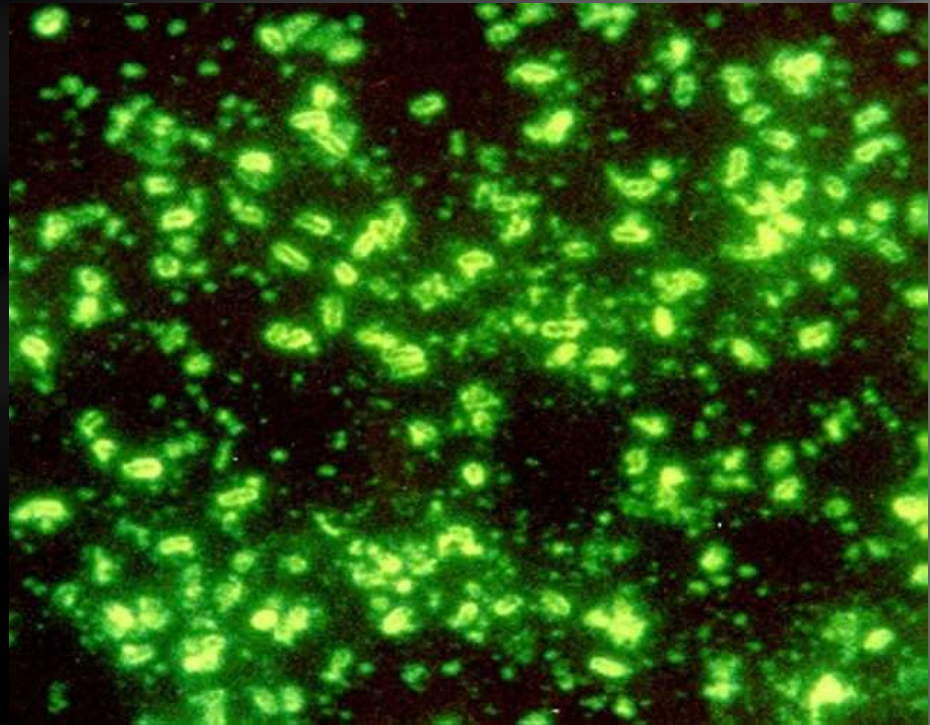
Ypcaf Operon

Background

- Vaccines
 - F₁ alone
 - protective to $<10^3$ LD₅₀'s in mice ag. 25C challenge
 - Protection increased by 2 logs in mice ag 37C challenge
 - V alone
 - protective to 10^5 LD₅₀'s in mice ag 25C challenge
 - Protection reduced by 70% in mice ag 37C challenge
- Dr. Rozak– F₁ and V antigen based vaccines
 - Investigated links between expression and observed immunity

Research Objectives

- Determine expression of Lcr V antigen
 - With F1 antigen
 - *Y. pestis* strain CO92
 - Without F1 antigen
 - *Y. pestis* strain C12



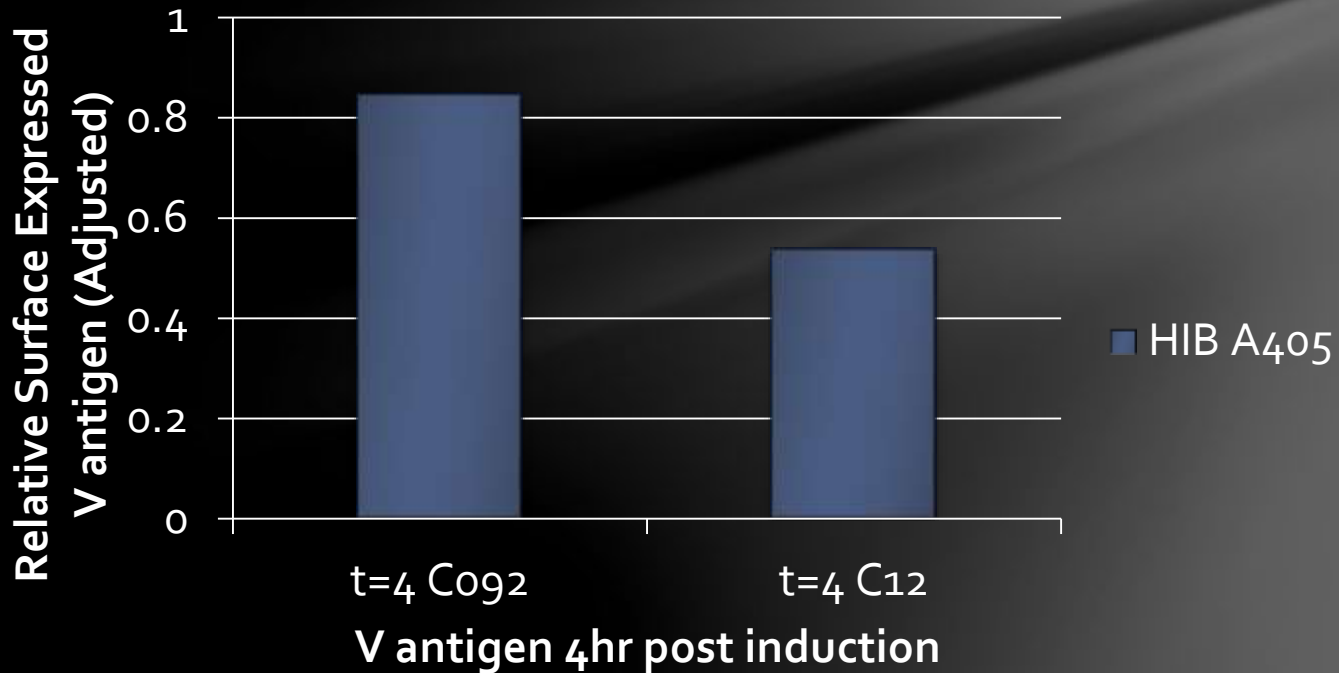
Methods

- Bacterial Growth
 - Strains: CO92 and C12
 - HIB Media
 - Over night culture at 25C
 - Shift to 37C and 25C culture
- SLIM Assay - Suspension-labelling Immuno[assay]
 - Anti-LcrV MAb
 - anti-mouse IgG, alkaline phosphatase conjugated
 - pmpp
 - A405

Results

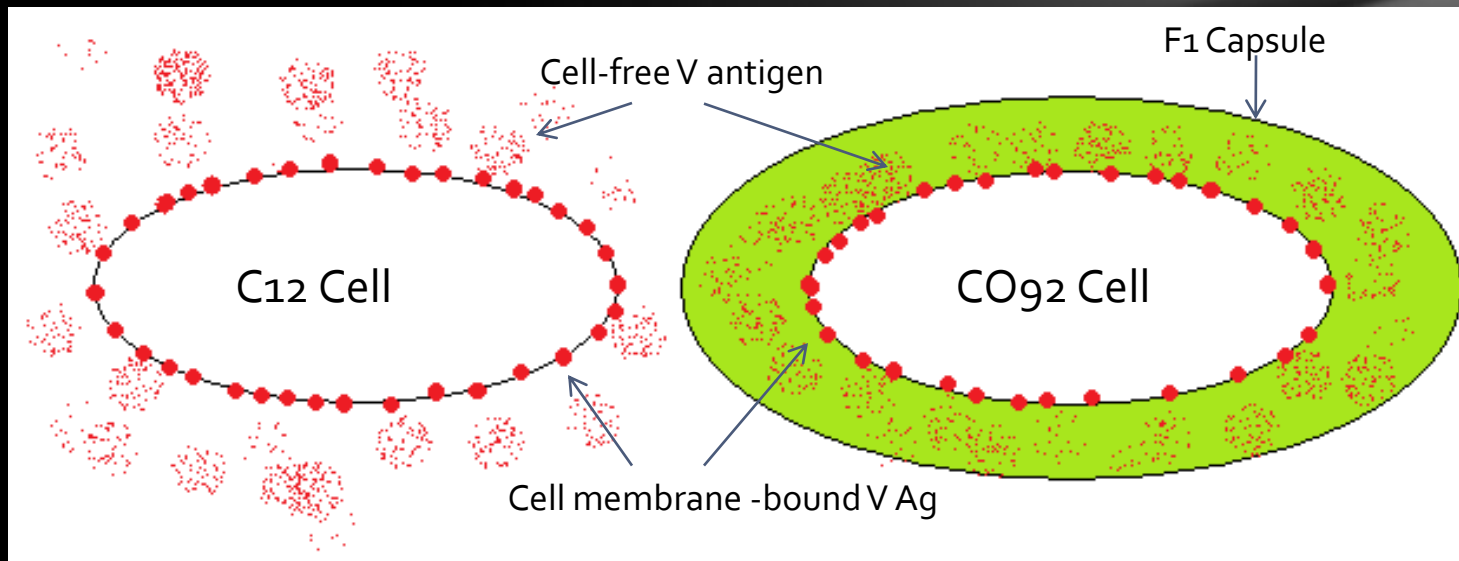
t=4 <i>Y. pestis</i> C092	
HIB Media	
OD ₆₀₀	1.42
A ₄₀₅	1.20

t=4 <i>Y. pestis</i> C12	
HIB Media	
OD ₆₀₀	0.68
A ₄₀₅	0.36



Results

- Goes against original hypothesis
 - Previously thought C12 would have more surface-exposed V
- Alternative hypothesis:
 - Cell-free V antigen “trapped” by F1 capsule



Conclusions

- *Y. pestis* causes plague
- Two different transmission modes
 - Effects of growing conditions (temperature)
 - Current vaccines impacted by these conditions
- Further testing required to expanded on preliminary results
- Future research being done this summer

Application

- Effective Vaccines
 - F₁ and V Combined as one hybrid protein ...“F₁-V”
 - protective to >10⁷ LD₅₀'s in mice ag. 25C and 37C challenge
 - IND status; in clinical trials
- Expand current knowledge-base

Acknowledgements

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