Yersinia pestis proteomics and vaccine development

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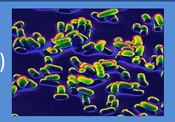
Pneumonic plague, caused by the gram-negative bacteria *Yersinia pestis*, has led to millions of human casualties during several major pandemics and has the potential to be employed as a deadly biological warfare agent. For this reason, it is vital that an effective vaccine against *Y. pestis* be created. Currently, a subunit vaccine exists which works by stimulating B-cells (humoral immunity), but it has failed to protect primates in aerosolized bacterial challenges. In an effort to complement the existing vaccine, an alternative vaccine strategy can use both B-cell stimulation and the targeting of T cells (cell-mediated immunity) with an antigenic peptide isolated from the bacteria. To determine what proteins may stimulate T cells, bacteria from three different strains of *Y. pestis*, KIM 5, KIM 6, and KIM 8, were cultured on TBA plates for four days and then in BCS liquid medium at either 26 or 37°C for four hours. Subproteomic fractionation of different regions of *Y. pestis* cultures were obtained and examined using 2D-DIGE, size-spin filters, and mixed-mode chromatography to isolate protein fractions that may stimulate T cells. Once differential proteins are selected, future work includes determining structure by mass spectrometry, and testing for antigenic peptides on T4 and T8 T cell stimulation assays.

Yersinia pestis

(a)



- Gram-negative bacteria (a)
- Lives in the stomach of fleas (b)
- Causative agent of plague.
- Most lethal pathogen in human history

(b)

- Three forms of infection:

(c)

- **Bubonic**
  - Swelling of the lymph nodes
  - Many epidemics throughout history (c)
- **Septicemic**
  - Infection of the blood
- **Pneumonic**
  - Infection of the lungs
  - Can be passed person to person through the air

- **Potential bioterrorism agent**
- Need to develop a safe and effective vaccine
  - Ineffective vaccines
    - Killed, whole cell do not offer adequate protection
    - Live attenuated vaccine not licensed for human use (3).
    - Subunit vaccine targeting **B-cells**, humoral immunity, has been ineffective in primate aerosol challenges (1).
    - New approach: make a vaccine that targets **T-cells**, cell-mediated immunity, in combination with the B-cell vaccine (2). (d)

(d)

- How? **Proteomics**- The collection of proteins an organism is expressing at any given time.
  - Catalog proteins and better understand pathogenicity in this organism
  - Detect specific proteins associated with virulence
  - Determine proteins that elicit T cell activity using different proteomic samples

Acknowledgements

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Works Cited

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(3) Titball, R.W. and Williamson, E.D. (2004) *Yersinia pestis* (plague) vaccines. Expert Opin.*Biol. Ther.* 4: 965-973.
(a): http://www.britannica.com/EBchecked/topic/462675/plague
(b): http://uhaweb.hartford.edu/bugl/histepi.htm
(c): http://bcm.bc.edu/issues/winter_2005/ll_plague.html
(d): http://nobelprize.org/educational/medicine/immunity/immune-detail.html

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Methods

Grow Bacteria
- *Y. pestis* cultured on TBA plate for 4 days
- In BCS liquid culture for 4 hours at 26 or 37°C

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5-part Subproteomic Fractionation
- Separate cell into 5 fractions

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TCA Prep
- Exchange sample buffer to desired buffer

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Protein Assay
- Determine protein concentration

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2D-DIGE: comparison of protein expression
- Labeling with dye
- Electrophoresis (charge and size separation)
- Image acquisition and analysis

↓

Collect proteins
- Mass Spectrometry to determine structure
- T4 & T8 T cells assays for vaccine development

↓

Mixed-Mode Chromatography
- Identify, Quantify, and Purify proteins

Table 1. Summary of samples used.

Three Strains of *Y. pestis*
- **KIM 5-** no pgm locus
- **KIM 6-** no pgm locus, no pCD1 plasmid
- **KIM 8-** no pgm locus, no pPCP plasmid

Two growing temperatures
- 26° C - flea body temperature
- 37° C - human body temperature

Five protein fractions of gram-negative bacteria
- Secretome (Sec)
- Outer membrane (OM)
- Periplasm (Peri)
- Inner membrane (IM)
- Soluble cytosolic (Sol Cy)

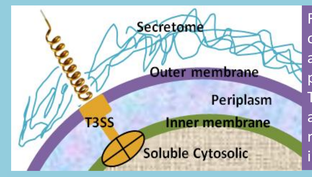

Fig.1. Schematic of cell fractions of *Y. pestis*. An ultracentrifuge is able to separate these layers for protein collection and testing. Type III Secretion System (T3SS) is an example of a pathogenic mechanism by which bacteria infect host cells.

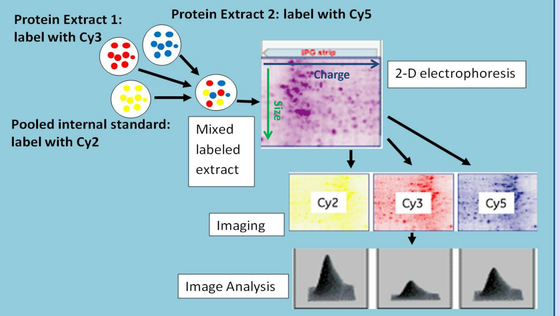

Fig. 2. How 2D-DIGE works.
Adapted from: http://www.gelifesciences.com

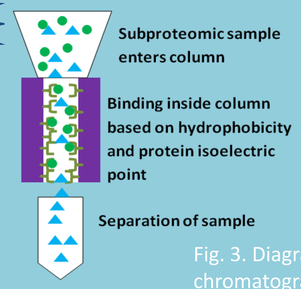

Fig. 3. Diagram of mixed-mode chromatography.

Proteomic Results

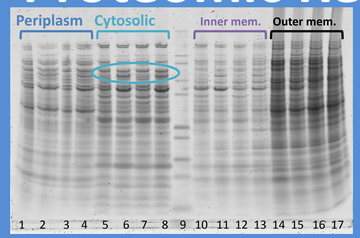

Fig.4. KIM5 and KIM6 Subproteomic Fractions.
1.KIM5 26°C Peri
2. KIM5 37°C Peri
3.KIM6 26°C Peri
4. KIM6 37°C Peri
5.KIM5 26°C Sol Cy
6. KIM5 37°C Sol Cy
7.KIM6 26°C Sol Cy
8. KIM6 37°C Sol Cy
9.Mark
10.KIM5 26°C IM
11. KIM5 37°C IM
12.KIM6 26°C IM
13. KIM6 37°C IM
14.KIM5 26°C OM
15. KIM5 37°C OM
16.KIM6 26°C OM
17. KIM6 37°C OM

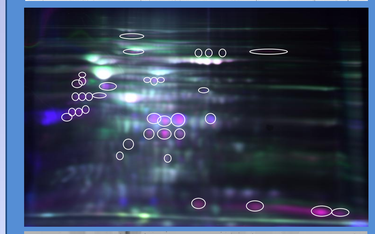

Fig.5. Periplasm fractions, 2D-DIGE, pH 3-10. Red spots-K537.Blue spots-K626.Green spots -K837.

Table 2. Differential Protein Expression

Comparison	Sample	Number of Increased Expression Spots
Cy2 to Cy3	KIM537	2
	KIM626	15
Cy5 to Cy3	KIM837	42
	KIM626	45
Cy2 to Cy5	KIM537	42
	KIM837	26

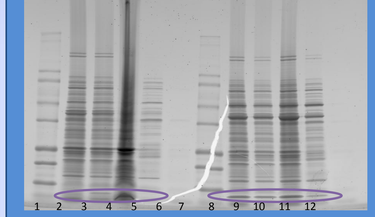

Fig.6. KIM5 at 26 and 37°C Periplasm fractions separated by size by size-spin filters.
1.Mark
2.K526 large tube
3.K526 small tube
4.K526 >100K
5.K526 100-20K
6.K526 <20K
7.Mark
8.K537 large tube
9.K537 small tube
10.K537 >100K
11.K537 100-20K
12.K537 <20K

Discussion

Different banding patterns in gels indicate differences in protein expression between the subcellular fractions, bacterial strains, or between temperatures. Confirmation of the protein differences between the subcellular fractions is shown in Figure 4. Between the bands that are circled notice how there are two bands at 37°C and only one band at 26°C in the same position. This indicates there is a difference in *Y. pestis* expression of proteins when the bacteria infects a host.

In Figure 5, the spots circled demonstrate periplasm proteins differentially expressed between the strains. Table 2 gives a comparison of increased protein expression comparing the strains. K537 and K626 appeared to be the most similar suggesting differences seen in K837 correlate with absence of the pPCP plasmid. Proteins differentially expressed when this plasmid is present suggest its crucial for up regulating proteins. These proteins will be subject to further study. Figure 6 show the differential expression of KIM5 periplasm proteins at 26 and 37°C. These proteins may be associated with virulence. These proteins are promising for additional testing as antigenic peptides for vaccine development.

Future work

- Periplasmic fraction has been shown to elicit a response in T-cells
- Further proteomic characterization research will be continued
- Additional samples will be sent to the Trudeau Institute for T cell testing
- Antigenic peptides will be determined to help develop a vaccine
- Vaccine including T cell epitopes will be tested to prevent *Y. pestis* infections
- Add vaccine to United States arsenal against bioterrorism