Monitoring Human Exposures to *Bacillus thuringiensis* after Aerial Applications

Bio-Weapons, Insects, and Humans

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Biological Control of Insects

- Microbial insecticides for control of insect pest populations: Biological Warfare

- Biological weapons of choice:
  - Bacteria: *Bacillus thuringiensis*
  - Viruses: Nucleopolyhedrovirus
Bacillus thuringiensis (Bt)

- Gram positive, spore-forming bacterium
- Ubiquitous in soil
Bacillus thuringiensis (Bt)

- Member of the *Bacillus Cereus*-Group of *Bacillus*
  - *Bacillus thuringiensis*
  - *Bacillus cereus*
  - *Bacillus subtilis*
  - *Bacillus mycoides*
  - *Bacillus anthracis*

- Very similar morphologically and biochemically

- *B. cereus* can cause gastroenteritis and diarrhea

- *B. anthracis* is highly pathogenic; Anthrax
Bacillus thuringiensis (Bt)

- Bt distinguished by presence of parasporal “Crystal Protein” = insect-specific toxin
Many different types of *cry* genes carried by different Varieties or “Subspecies” of *Bt*

- *Bt* var. *Kurstaki* (*BtK*) - specific to Lepidoptera
- *Bt* var. *Israeliensis* (*BtI*) - specific to Diptera

Many Strains within Varieties

- *BtK* HD1 encodes a complex of five Lepidoptera-specific toxin genes: *cry* 1Aa, *cry* 1Ab, *cry* 1Ac, *cry* 2A, and *cry* 2B
The Gypsy Moth

- The gypsy moth- *Lymantria dispar*:
  - Major deciduous tree defoliating insect pest in eastern North America
  - Not yet established in British Columbia
  - Infestation became problematic, 1998 - 1999
  - Sale and export of B.C. lumber products, valued at approximately $2.7 billion dollars, faced embargo if gypsy moth populations were not controlled
Gypsy Moth Control in Victoria

- Population of Victoria is approx. 70,000

![Map of Victoria with aerial spraying areas marked with red dots and direction of prevailing winds during the time of aerial applications of Foray 48B.]
Gypsy Moth Control in Victoria

- How was spray applied?
Gypsy Moth Control in Victoria

- Molasses (20% solution) is used as carrier
Aerial Application of Foray 48B

- Microbial insecticide, Foray 48B contains: *B. thuringiensis* subsp. Kurstaki, strain HD-1

- Spray applied by Cessena 188:
  - 580 L @ 70 L/min (approx. 152 gal @ 20 gal/min)

- Applied at 4 L / hectare
  (0.25 gal/acre)

- Droplet size: 110 – 130 µm
Aerial application of Foray 48B

- Applied by aircraft in 3 spray periods

  - 1st Spray, May 09, 10
  - 2nd Spray, May 19, 20, 21
  - 3rd Spray, June 08, 09
Bt Safety

- *BtK* is not toxic to mammalian species
- Toxin is quickly degraded in the environment by UV-light
- Despite safety, concern over possible health impact of aerial spraying mandated public health study
Health Impact Study

- To determine if bacteria isolated from air, human, food, and water samples collected pre- and post-spray were *BtK HD1*

- Environmental samples collected pre- and post-spray from Air, water, and grocery produce pre- and post-spray

- Nasal swabs collected from 15 families (approx. 50 people) pre- and post-spray

- Samples collected both inside and outside spray zone
Bacteria within *B. cereus*-group are very similar biochemically and morphologically.

*B. thuringiensis, B. cereus, and B. anthracis* are closely related at both nucleic acid and amino acid levels.
- *B. thuringiensis* and *B. cereus* have very similar genome organization
Technical Challenge

- *B. thuringiensis*, *B. cereus*, and *B. anthracis* considered to be variants of the same “species” differentiated only by presence of specific plasmids which encode toxins

- *B. thuringiensis*: *cry* gene plasmids

  - *BtK HD1* encodes a complex of five toxin genes encoded on three plasmids:
    - *cry 1Aa, cry 1Ac*
    - *cry 1Ab*
    - *cry 2A, cry2B*
Experimental Approach

- Combined use of three molecular techniques to identify *BtK* HD1 in exposed and non-exposed individuals
  - RAPD- PCR
    - Random Amplified Polymorphic DNA- Polymerase Chain Reaction
  - *Cry*-gene PCR
  - Dot Blot Hybridization

- Amplification of genomic DNA from *BtK* HD1 produced four characteristic bands of approximately 1000, 800, 60, and 400 bp
RAPD-PCR

- Able to distinguish *BtK* from several varieties of *Bt* and between *BtK* and *B. cereus*
RAPD-PCR of Different *Bt* Varieties

-ve Control *B. cereus*
RAPD-PCR From Nasal Swabs

BtK HD1:

Low frequency (2.8%) of additional bands; Confirmed +ve by cry Gene PCR

-ve Control B. cereus

+ve Control BtK HD1
Cry 1 Gene PCR

- *BtK* HD1 contains 3 plasmids that encode complex of five toxin genes:
  - cry 1Aa, cry 1Ab, cry 1Ac, cry 2A, and cry 2B
  - Presence of cry 1Aa, cry 1Ab, cry 1Ac is diagnostic of *BtK* HD1

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<tr>
<th></th>
<th>cry1Aa</th>
<th>cry1Ab</th>
<th>cry1Ac</th>
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<td>Size (bp)</td>
<td>1,500 bp</td>
<td>858 bp</td>
<td>653 bp</td>
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Cry 1 Gene PCR

BtK HD1:

-ve Control B. cereus

+ve Control BtK HD1

1 Kb

1500 bp

858 bp

653 bp
Limitations of PCR-Based Analyses

- RAPD and *cry* gene PCR are very sensitive
  - Extremely low frequency of False Positives
  - High frequency of False Negatives (32%)

- Confirmed PCR-based results by DNA Hybridization
  - Low frequency of False Negatives (< 2%)
  - May produce some False Positives
Cry 1 Gene Hybridization

- Screened 171 isolates of bacteria from nasal swabs and 29 isolates from food samples

-c = B. cereus
+c = BtK HD1
Controls
-c = B. cereus
Cry 1 Gene Hybridization

- Screened over 10,000 isolates of bacteria from air samples: 85.4% BtK HD1 Positive
Combined Analysis
Nasal Swabs- First Spray

Pre-Spray: 47.0 % *BtK* HD1  Post-Spray: 84.8 % *BtK* HD1
Combined Analysis

Nasal Swabs - Second Spray

Pre-Spray: 77.7% *BtK* HD1   Post-Spray: 82.3% *BtK* HD1
Combined Analysis
Nasal Swabs- Third Spray

Pre-Spray: 64.0 % \textit{BtK} HD1  
Post-Spray: 84.1 % \textit{BtK} HD1
Conclusions

- Positively identified *BtK* HD1

- Distinguish *BtK* HD1 from other varieties of *Bt* and from different isolates of *B. cereus*

- *BtK* HD1 was present in environment and human population of Victoria prior to aerial applications of Foray 48B

- Incidence of *BtK* HD1 increased human population even though people were inside houses at time of spray