Fate and effect of *Bacillus thuringiensis* based insecticides in the human gut

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Background

- *Bacillus thuringiensis (Bt)* widely used as a bio-insecticide
- Produces enterotoxins that causes diarrhoea in humans
- *Bt* is sprayed as spores on the plants, but unclear whether the spores germinate in the human intestine
\textbf{B. thuringiensis in food}

- Grapes
  - Bidochka et al, 1987; Bae et al, 2004
- Farm bulk tank, creamery silo tank
  - Phillips et al, 1986
- Pasta, bread, milk, fruit, vegetables
  - Damgaard et al, 1996; Rosenquist et al, 2005
Objectives

• Do ingested spores and vegetative cells of *Bt* survive and persist in the gut?
• Do ingested spores germinate in the gut?
• Does heat-activation of spores have an influence on their survival, persistence and germination?
• Do ingested *Bt* cells change the composition of the indigenous microflora of the gut?
• Are we able to detect enterotoxins in the gut?
Investigated strains

- *Bacillus cereus* F4433/73 – RifR
- *B. thuringiensis* subsp. *kurstaki* DMU67R – RifR
- *B. thuringiensis* subsp. *isralensis* 4Q1 - StrR
Animal experiments

- Group 1: Irradiated spores
- Group 2: Untreated spores
- Group 3: Heat-activated spores
- Group 4: Vegetative cells

Human flora associated rats

- Faecal and intestinal samples

- Plate counts
- DGGE
- Enterotoxins (NERI)
**B. cereus F4433/73** in faecal samples

- **Untreated spores**
- **Heat-activated spores**
- **Dosage untreated spores**
- **Dosage heat-activated spores**

**Graph Details:**
- **Y-axis:** log cfu/g faeces
- **X-axis:** Time/days
- **Legend:**
  - Blue line for Untreated spores
  - Red line for Heat-activated spores
  - Cross for Dosage untreated spores
  - Cross for Dosage heat-activated spores

**Day of sacrifice** indicated at the bottom right of the graph.
Bc/Bt counts in intestinal samples at Day 5

- **B. cereus**
- **Btk**
- **Bti**

**log cfu/g intestinal content**

- Colon
- Caecum
- Ileum
- Duodenum
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DGGE with universal primers on faecal samples from HFA rats fed *B. cereus* F4433/73R

a: *Ruminococcus obeum*  
b: *Fusobacterium sp.*  
c+d: unculturable bacterium

DGGE – PCA analysis
Rats fed *B. cereus* F4433/73 spores

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Summary (1)

- *B. cereus* and *Bt* cells can be detected in faecal and intestinal samples up to two weeks after last dosing (multiplication, attachment)

- One animal fed *Bt* subsp. *kurstaki* had high levels of *Bt* subsp. *kurstaki* in the intestine and faeces after two weeks
Summary (2)

• Vegetative cells are presumably killed in the stomach, and hence no or only few cells are found in faecal and intestinal samples.

• No difference in survival and persistence in the GI tract of heat-activated and untreated spores.

• Shift in bacterial population after feeding with *B. cereus* F4433/73.
Conclusion

• *B. thuringiensis* insecticidal strains can be isolated from food products
• *Bt* spores may germinate, grow, re-sporulate in the gut, and some may be able to pass the GI barrier and reach other organs (host factor, immune status, gut microflora)
• Rats are not good models for assessing the virulence of *Bt* for humans
Acknowledgements

Danish Institute for Food and Veterinary Research
Bodil Madsen
Kristine Frederiksen
Hanne Rosenquist
Tine Rask Licht

National Institute of Occupational Health
Lasse Smidt
Lars Andrup

National Environmental Research Institute
Bjarne Munk Hansen
Niels Bohse Hendriksen

Danish Environmental Protection Agency