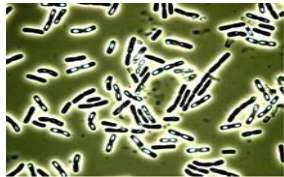


Biosafety and biology of *Bacillus thuringiensis*
a response to the EFSA opinion

Dr Ben Raymond

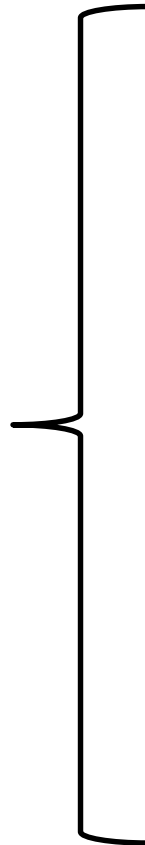
Introduction to the *Bacillus cereus* group

Bacillus cereus sensu lato



Gordon et al 1973
DNA re-association work & 16SrRNA
indicate homogeneity

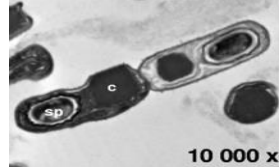
*Virulence factors on plasmids



*B. anthracis** (note capsule)



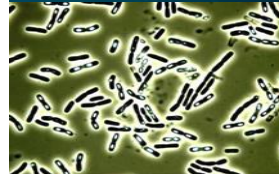
B. cereus sensu stricto



*B. thuringiensis**



B. mycooides



B. weihenstephensis
(psychrotolerant)

Importance of taxonomy- is *Bt* the same as *B. cereus* or not?

Food industry testing for “presumptive *B. cereus*” is very general.

Tests do not distinguish *B. cereus sensu stricto* from “less commonly encountered *B. weihenstepanensis*, *B. thuringiensis*, *B. mycoides*”

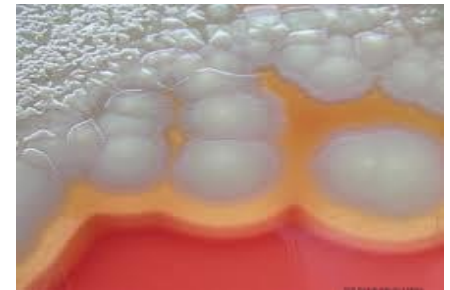


ISO 7932:2004

Standard tests



Pink colonies lecithinase positive on MYP agar



haemolysis on sheep blood

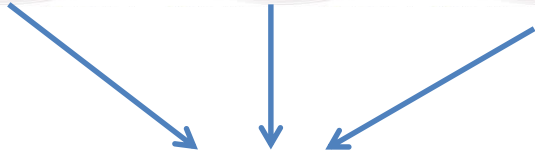
The data from the original food poisoning event



6×10^3 CFU g⁻¹
B. cereus



$\approx 1 \times 10^5$ CFU g⁻¹
B. thuringiensis



Diarrhoea

No faecal samples

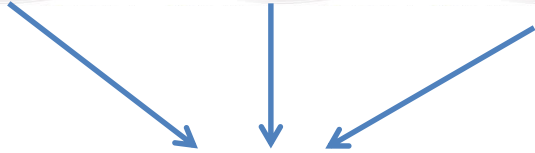
The data from the original food poisoning event



6×10^3 CFU g⁻¹
B. cereus



$\approx 1 \times 10^5$ CFU g⁻¹
B. thuringiensis



Diarrhoea

Probability that infected individuals
ate salad by coincidence

$$= 5! / (3!(5-3)!) = 0.1 = \mathbf{1/10}$$

Nevertheless....this food poisoning event led to a call by EFSA for re-examination of the data on biosafety of *B. thuringiensis*....

SCIENTIFIC OPINION



ADOPTED: 9 June 2016

doi: 10.2903/j.efsa.2016.4524

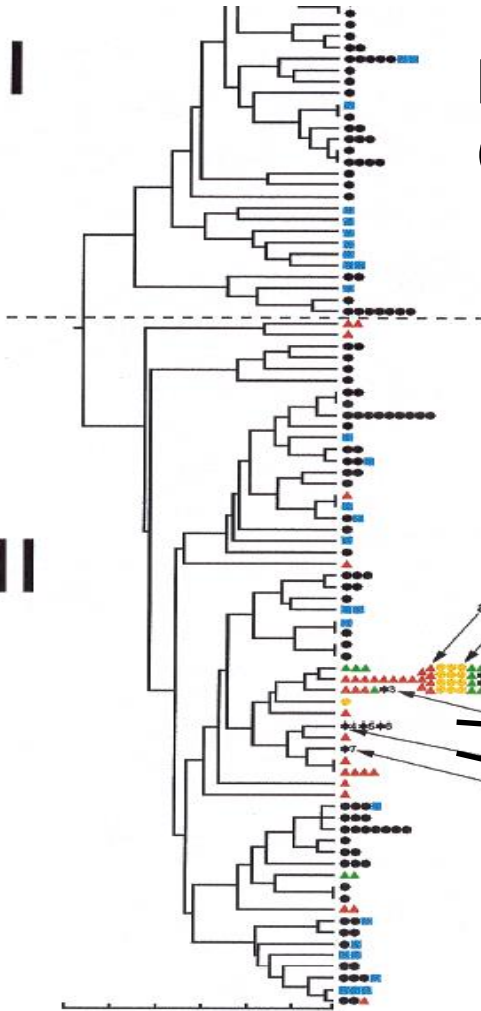
Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs

EFSA Panel on Biological Hazards (BIOHAZ)



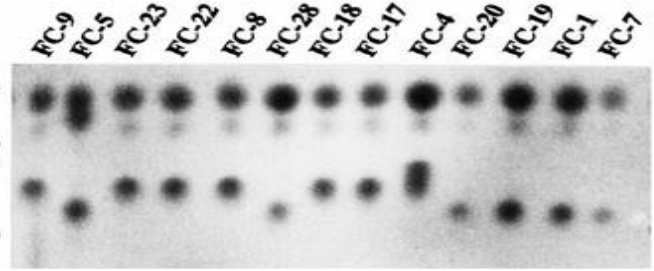
Key Suppositions of the EFSA Opinion

- *B. cereus* is “the same” as *B. thuringiensis*
- *B. thuringiensis* is as dangerous as *B. cereus sensu stricto* for humans
- Previous studies have shown *Bt* has been associated with acute human infections
- Previous studies have shown *Bt* has been “involved with” food poisoning “
- We don’t much about safety of *Bt* – there may be “cryptic” unrecorded infections.....



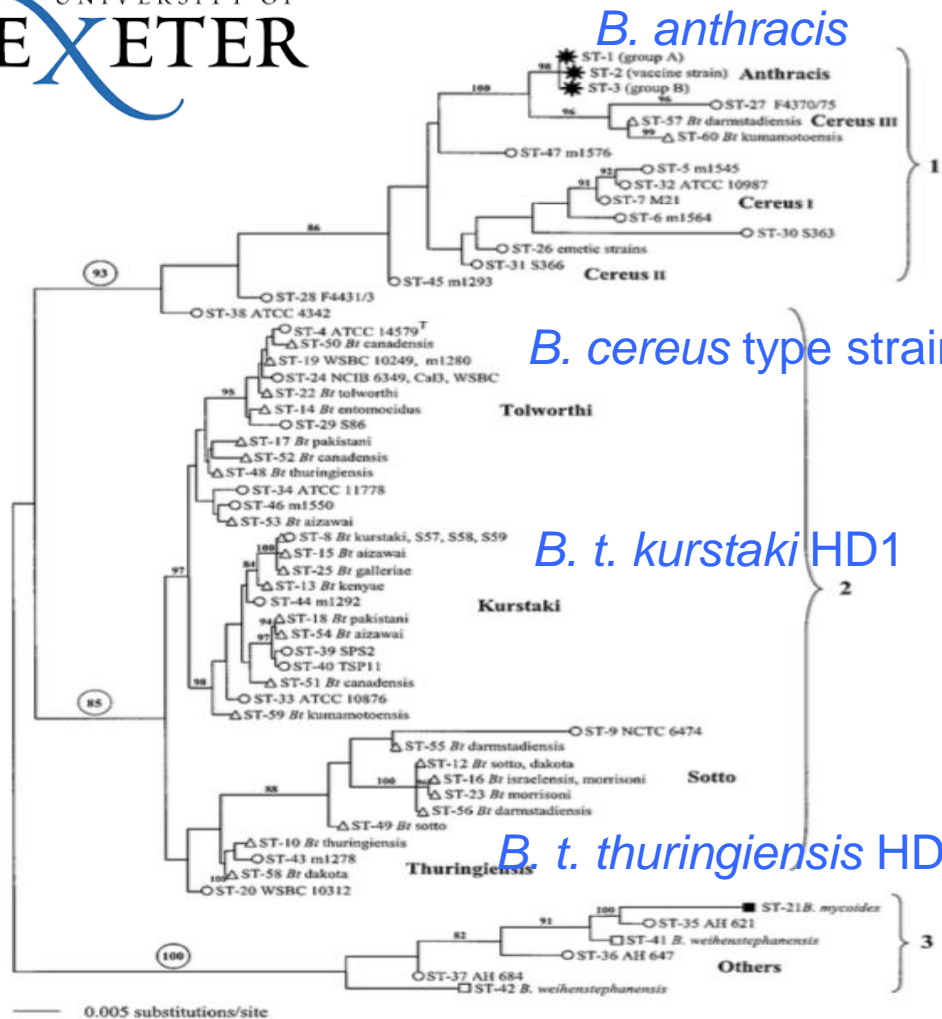
Early studies perpetuate idea of homogeneity

(based on MLEE - starch gel protein electrophoresis)



- B. anthracis*
- B. t. thuringiensis* HD2
- B. t. kurstaki* HD-1
- B. cereus* type strain

Helgason et al 2000..”one species on the basis of genetic evidence”



Multilocus sequence typing (MLST) presents different picture

Priest et al 2004-- 2838bp DNA

Clade 1- “anthracis” clade

	. *. * * * * : . . . ** * : * : * * : * . **** *
KT376737	AAACGGACATTTTTCCTTTAGAAACAAGGATACGTATAACGTCGATMGGCCTTTGT
KT376740	AAAAGGACATTTTTCCTTCCGTAAAGCGG-TCCGTAATAACGAAGATMGGTCTTTGTT
KT376736	AAACGGATATTTTTCCTTTGAGAGTAGGGG-TCCGACATAACCTCGATGATCTTTGT
KT376742	AAACGGAAATTTTTCCTTACGTAAGGTTG-CGGTAATAACGTTGATGGTCTTTGT
KT376739	AAACGGAAATTTTTCCTTACGTAAGGTTG-CGGTAATAACGTTGATGGTCTTTGT
KT376741	AAACGGATATTTTTCCTTACGTAAGGTTG-CGGTAATAACGTCGATGGGCTTTGT
KT376743	AAACGGACATTTTTCCTTTGCTAAGCGG-TCCGTAATAACGTCGATCGGCTTTGT
KT376738	AAACGGACATTTTTCCTTTGCTAAGCGG-TCCGTAATAACGTCGATCGGCTTTGT
KT376744	AAAGGGCAGTTTTCCTTTGCTAAGCGG-TCCGTAATAACGTCGATCGGCTTTGT

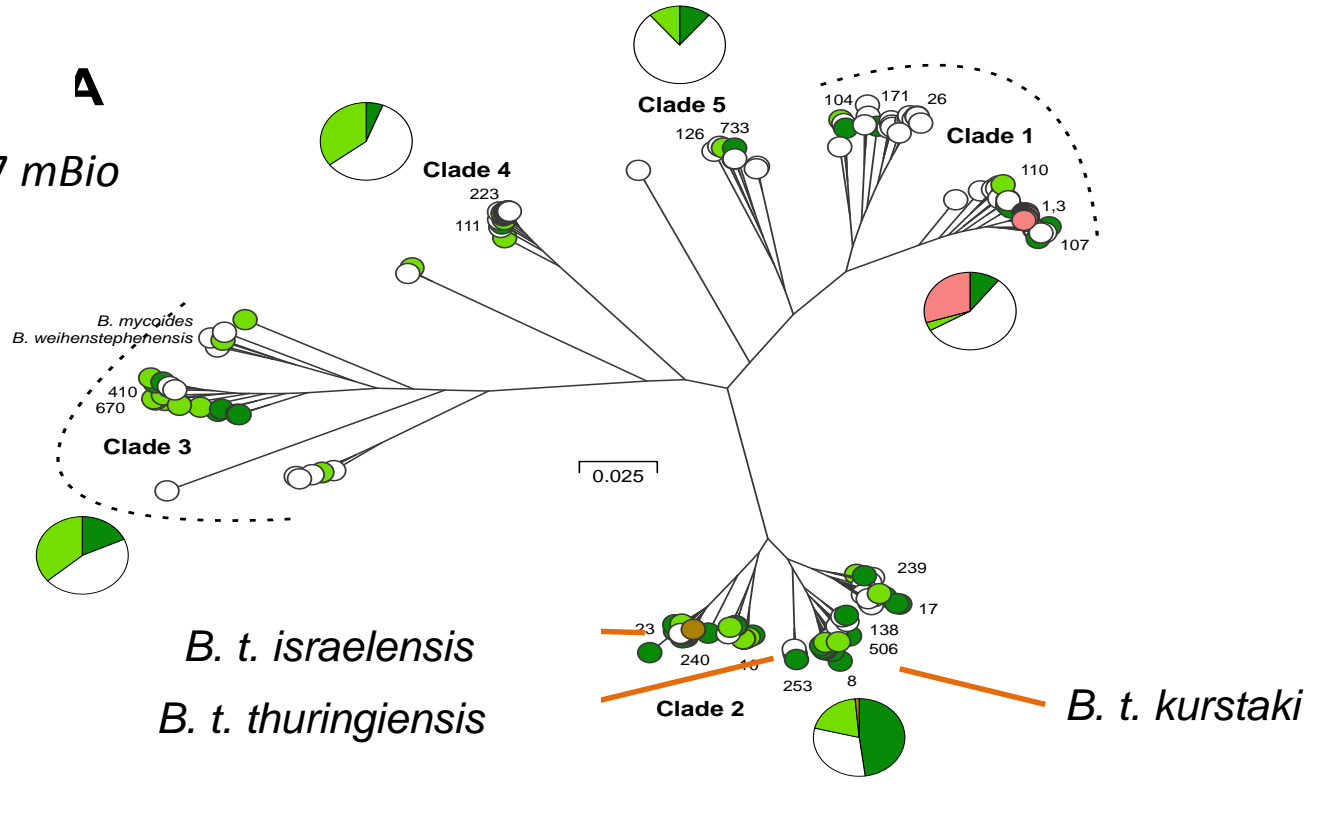
Clade 2- “kurstaki” clade

Clade 3- “weihenstephanensis” clade

And now with whole genome data---showing distribution of Cry toxins across clades

Méric et al (in review)

see also Zheng et al 2017 *mBio*



Suppositions of the EFSA Opinion

- *B. cereus* is “the same” as *B. thuringiensis*

No, the *B. cereus* group is **genetically and ecologically heterogeneous**.

- *B. thuringiensis* is as dangerous as *B. cereus sensu stricto* for humans
- Previous studies have shown *Bt* has been associated with acute human infections

Ecological differentiation in the *Bacillus cereus* group

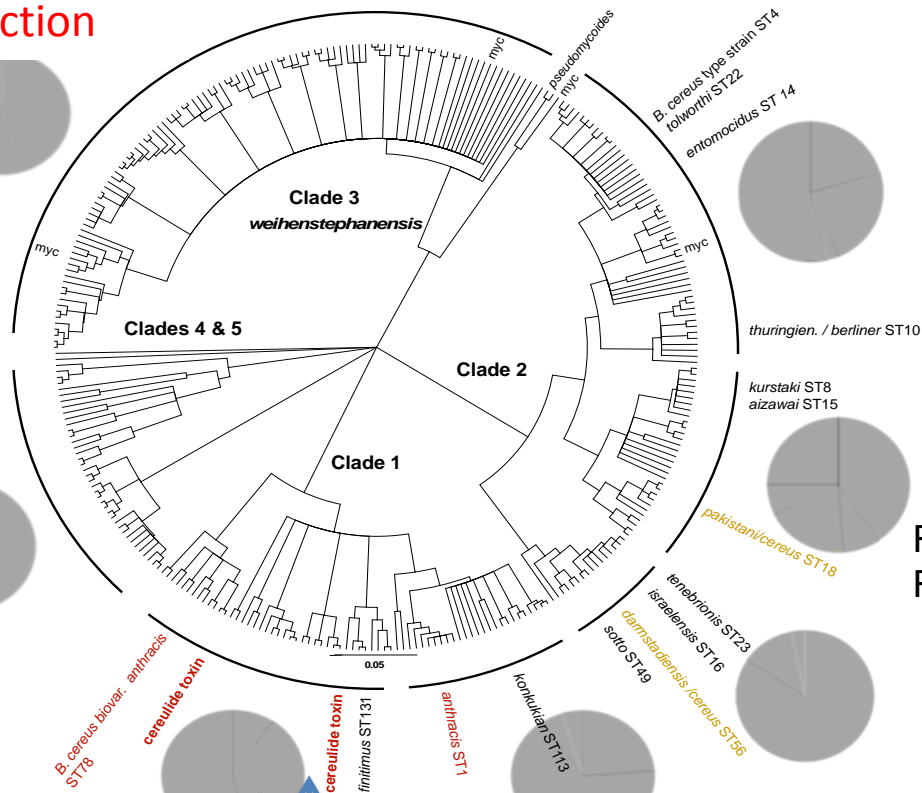
Vertebrate
infection

Cry toxin

Plant origin

Water

Faeces / Food poisoning



cereulide producers

invertebrate pathogens
- mostly in clade 2

vertebrate pathogens
- mostly in clade 1

Raymond & Bonsall (2013) Bioessays 35:705-6
Raymond & Federici (2017) FEMS Microbiol Ecol

Ecological differentiation in the *Bacillus cereus* group

Ability to colonize plants

(Vidal-Quist et al 2013, FEMS Microb Ecol
Raymond et al 2010, PloS Path)

Distribution in environment / assoc with infection

(Raymond et al 2010, Plos Path; Raymond & Bonsall 2014
Guinebretière et al 2007 Env Micro)

Optimal growth temperatures

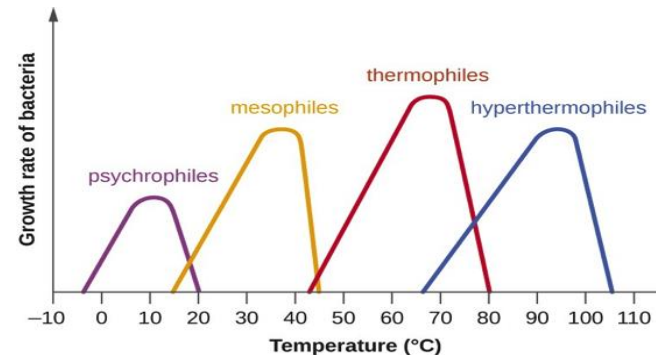
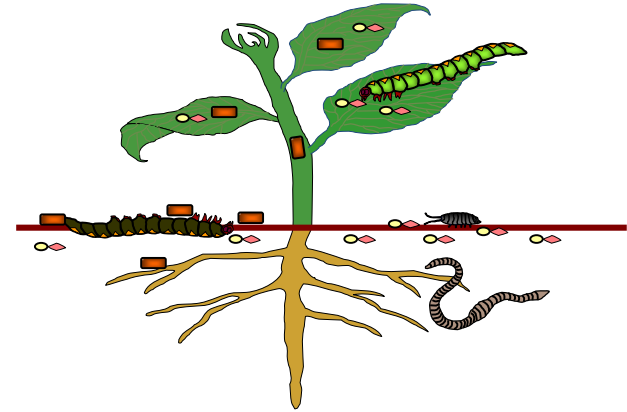
(Guinebretière et al 2007 Env Micro)

Ability to grow in insects

(Manktelow & Raymond SIP presentation 2017)

Toxicity to vertebrate cells

(Guinebretière et al 2010, J Clin Microbiol)



Suppositions of the EFSA Opinion

- *B. thuringiensis* is as dangerous as *B. cereus sensu stricto* for humans
Not true. Clinical database and exhaustive safety trials suggest otherwise
- Previous studies have shown *Bt* has been associated with acute human infection

Misleading if not actually wrong.

Bt in clade 1 (*anthracis*- like clade) has been associated with serious human infections (*very rarely*). All biopesticides are in clade 2.

Suppositions of the EFSA Opinion

- Previous studies have shown *Bt* has been “involved with” food poisoning “

Vague language here!- *Bt* is very widespread in food (biopesticides & natural strains)

Once possibly recovered from diarrhoea but associated with Norwalk virus

No evidence of causation

Suppositions of the EFSA Opinion

What about “cryptic infections”????

> **2000 isolates** in clinical MLST databases – no genotypes characteristic of biological pesticides have been recovered from human infections....

Product	serovar	MLST genotype	Isolates with this genotype in database	Clinical infections with this genotype
DiPel	<i>kurstaki</i>	ST 8	79	0
XenTari / Florbac	<i>aizawai</i>	ST 15*	8	0
Tekar / Vectobac	<i>israelensis</i>	ST 16	23	0
Novodor	<i>morrisoni</i>	ST 23	21	0

Data from puMLST : <http://pubmlst.org/bcereus> &
SuperCAT databases <http://mlstoslo.uio.no>

Conclusions

PERSECTIVE

In defence of *Bacillus thuringiensis*, the safest and most successful microbial insecticide available to humanity—a response to EFSA

Ben Raymond^{1,*†} and Brian A. Federici²

¹Environmental Sustainability Institute and Department of Biosciences, University of Exeter, Penryn Campus, Penryn TR10 9FE, UK and ²Department of Entomology and Institute for Integrative Genome Biology, University of California, Riverside, Riverside, CA 92521, USA

- Specialized invert pathogens are not harmful to humans (these are in clade 2)
- Human associated *Bt* strains & emetic *B. cereus* are in clade 1
- No biopesticide genotypes have every been recovered from clinical infections
- *Bt* probably can't cause diarrhoea because investment in Cry toxins makes it uncompetitive in gut

Acknowledgements

Brian Federici

Mike Bonsall, Kelly Wyres, Neil Crickmore

Sam Sheppard & Dr Guillaume Méric group at University of Bath (& collaborators)



The Leverhulme Trust

