Bacillus thuringiensis and food safety

Prof Ben Raymond
January 2023
SCoPAFF Webinar

UNIVERSITY OF EXETER · LUCEM SEQUIMUR ·
University of Exeter

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. Federici[2]

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

[*]**Corresponding author:** Environmental Sustainability Institute and Department of Biosciences, University of Exeter, Penryn Campus, Penryn TR10 9FE, UK. Tel +44 01326259085; E-mail: b.raymond@exeter.ac.uk
One sentence summary: This article critically examines the available evidence on whether *Bacillus thuringiesis*, a species commericially important in biocontrol, is capable of infecting vertebrates.
Editor: Marcus Horn
[†]Ben Raymond, http://orcid.org/0000-0002-3730-0985

218 bio control scientists agree with us: https://docs.google.com/spreadsheets/d/1oH-qLfVxgk9XjyEiDWZ89jPKxVOqznKZDKN8RBZpuBs/edit? usp=sharing).

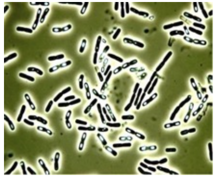
Suppositions of the EFSA Opinion

- *B. cereus* is "the same" as *B. thuringiensis*
- *B. thuringiensis* is as dangerous as *B. cereus sensu stricto* for humans
- We don't know much about safety of *Bt &* there may be "cryptic" unrecorded infections……
- Previous studies have shown *Bt* has been associated with acute human infections
- Previous studies have shown *Bt* has been associated with diarrhoea

University of Exeter

Introduction to the *Bacillus cereus* group *ca.* 1997

Bacillus cereus **group**

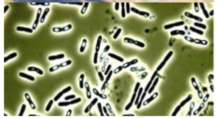


'Opportunistic' pathogen; spores widely distributed in terrestrial & aquatic habitats

Genomes specialized for protein / amino acid rich diet / lots of cellulytic enzymes

Carnivorous! Common niche is the ability to exploit dead bodies (necromeny)

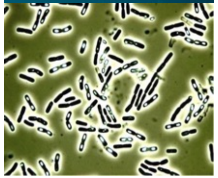
B. anthracis (note capsule)



B. cereus sensu stricto

B. thuringiensis (note Cry toxin inclusion)

B. mycoides



B. weihenstephanensis (cold adapted)

Suppositions of the EFSA Opinion

- *B. cereus* is "the same" as *B. thuringiensis*

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

University of Exeter

Bacterial species concepts and genetic distances

”Honey, I brought home a ..cat.. for the children to play with.”

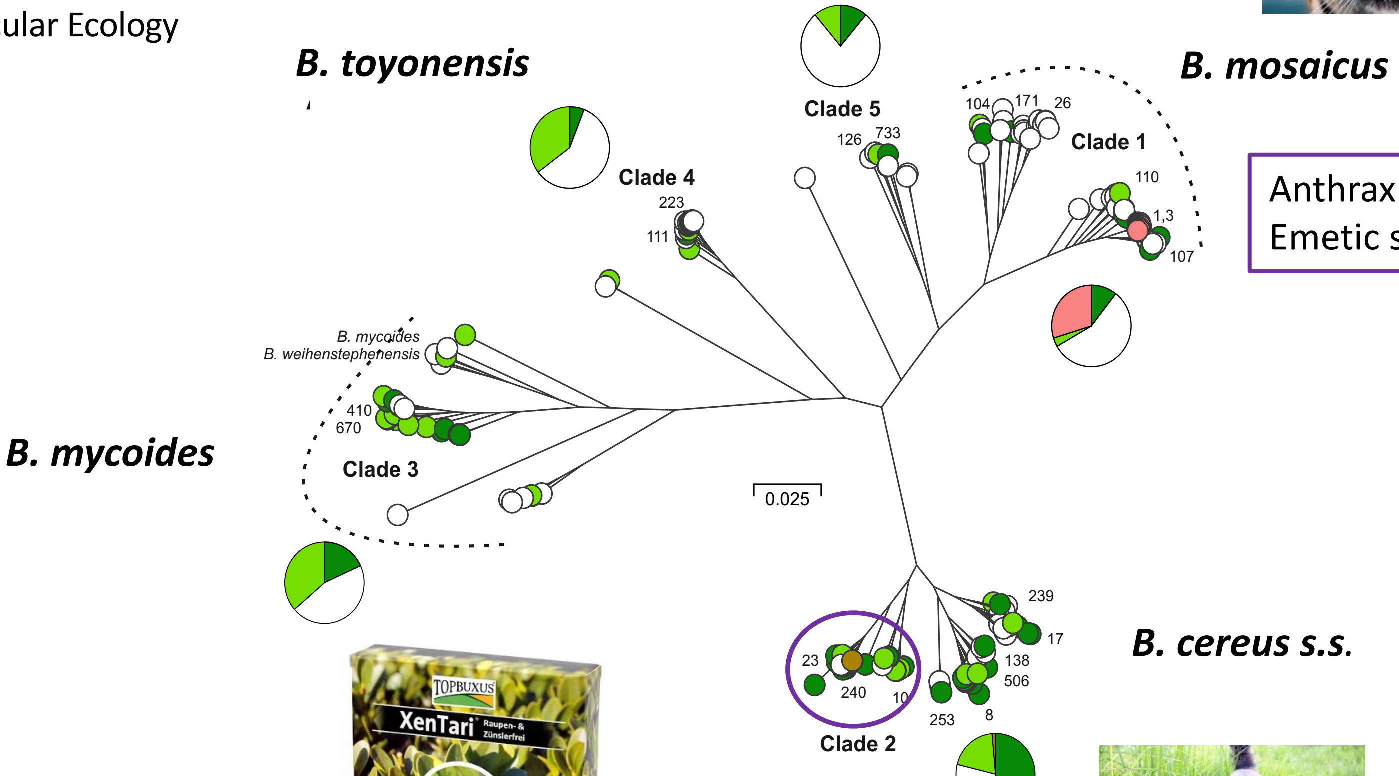
”Honey, I brought home a ..tiger.. for the children to play with.”

Tiger DNA sequences are around **5%** different from those of domestic cats. Sung-Cho et al (2013)

This is similar to the genetic differences used to separate bacterial species.

New species names suggested by Carroll et al 2020

Méric et al 2018 Molecular Ecology

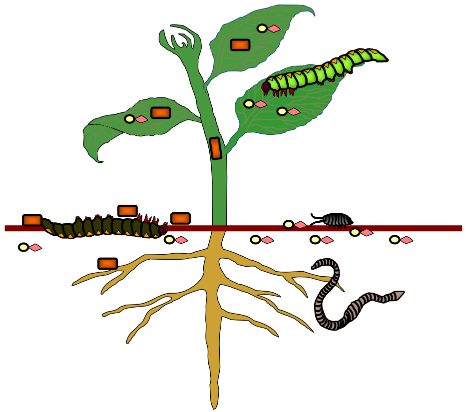


Anthrax
Emetic strains

No toxins
Candidate Cry
Cry positive (Bt)
B. anthracis

Biological differences between major clades or biovars in *B. cereus* group

Ability to colonize plants (Vidal-Quist et al 2013, FEMS Microb Ecol; Raymond et al 2010, PloS Path)



Biogeography. (Drenowska et al 2020)

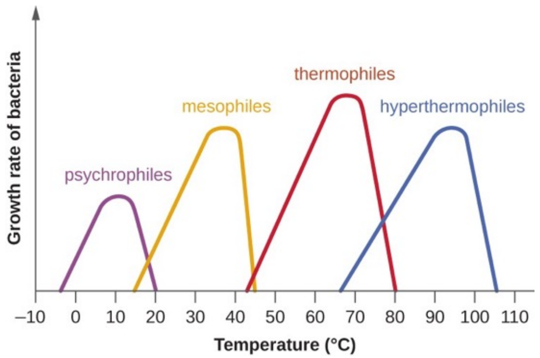
Optimal growth temperatures (some clades)
*(*Guinebretière et al 2008 Env Micro)

Ability to grow in insects / evidence of clade specific adaptation
(Manketelow et al 2020; White et al 2022)

Carriage of virulence genes (Cardazzo et al 2008)

Bt/Bc Replication in the gut (Raymond et al Science 2012;Raymond et al JIP 2007)

Toxicity to vertebrate cells; association with infection**
(Guinebretière et al 2010, J Clin Microbiol; Raymond et al 2010, Plos Path; Raymond & Bonsall 2014; Raymond & Federici 2017)



There may be "cryptic" unrecorded infections……"cryptic Bt infections"??

- **2000 isolates** in two clinically biased 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	*kurstaki*	ST 8	79	0
XenTari / Florbac	*aizawai*	ST 15	8	0
Tekar / Vectobac	*israelensis*	ST 16	23	0
Novodor	*morrisoni*	ST 23	21	

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

The aim of these databases
genotype infections!

Suppositions of the EFSA Opinion

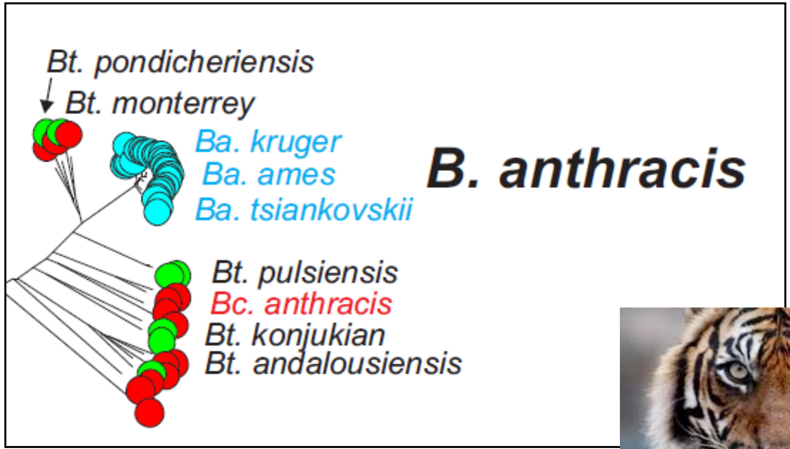
- Previous studies have shown *Bt* has been associated with acute human infection

Misleading if not actually wrong.

Cry toxin expressing strain in *"B. mosaicus"* have been associated with serious human infections *(very rarely*). These are distantly related to biopesticide strains

'Bt' konkukian- isolated from soldier injured by a mine; can kill mice superinfected with influenza Hernandez et al 2000 FEMS Immun Med Micro

Many would now classify as a different species



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
Doses of 10^7 - 10^8 Bt spores are asymptomatic for vertebrates

- Previous studies have shown *Bt* has been associated with diarrhoea

-Association means very little - Bt is very widespread in food (biopesticides & natural strains)
-typically associated with other pathogens
occasionally recovered from diarrhoea stools– but no evidence of causation

Bonis et al (2021) Found widespread Bt in food. Did not look at faecal samples. <u>BUT STUDY LACKED A CONTROL GROUP</u>

SUMMARY

- The *B. cereus* groups is diverse: the ecology varies substantially with clade
- Specialized invert pathogens are not harmful to humans
- Human associated “*Bt*” strains & emetic strains should be classified as *B. mosaicus*
- No biopesticide genotypes have every been recovered from clinical infections although they are widespread in food.

UNIVERSITY OF EXETER · LUCEM SEQUIMUR ·

University of Exeter

Acknowledgements

Students: James Manketelow, Hugh White, Kelly Wyres,

Collaborators:
Profs Brian Federici, Mike Bonsall, Neil Crickmore

Prof Sam Sheppard & Dr Guillaume Méric

The Leverhulme Trust

bbsrc
biotechnology and biological sciences
research council

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 ….
but no faecal samples

UNIVERSITY OF EXETER

The data from the original food poisoning event

$6x10^3$ CFU g^{-1}
B. cereus

$\approx 1x10^5$ CFU g^{-1}
B. thuringiensis

Diarrhoea ….
but no faecal samples

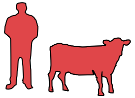
Probability that ***Bc*** infected individuals all ate salad by coincidence

$=5!/(3!(5–3)! = 0.1 =$ **1/10**

UNIVERSITY OF EXETER

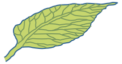
Ecological differentiation between *Bc* group species a pictorial review

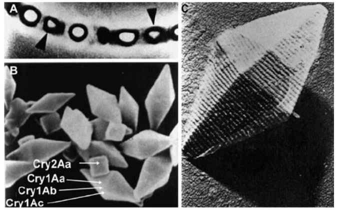
Clade 1 / *“B. mosaicus”*



Cereulide / anthrax toxins
Vertebrate toxicity

Clade 2 / *“B. cereus s.s.”*
& B. thuringiensis





Insecticidal toxins
(50% genomes)

Clade 3 / *B. mycoides*

Guinebretiere et al 2008, 2010; Raymond & Bonsall 2013; Méric et al 2018; Drenowska et al AEM 2020; Manktelow et al 2021 FEMS Microb Ecol