All about *Bacillus thuringiensis* (BT)

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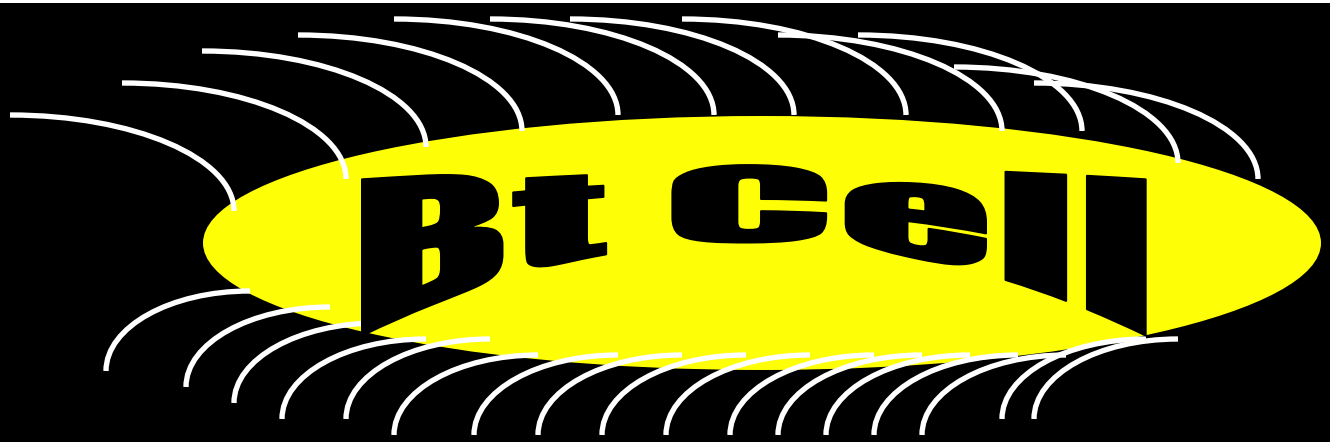
A spore-forming bacterium
in the genus Bacillus,
part of the Bacillus cereus group

Bacillus thuringiensis

> 50 subspecies, 3 commonly used

A biorational or microbial insecticide



Peritrichious: Multiple flagellae to swim. Subspecies based on the protein composition of the flagellae

- Bacillus thuringiensis subsp. kurstaki: H 3a, 3b, 3c
- Bacillus thuringiensis subsp. aizawai: H 7
- Bacillus thuringiensis subsp. israelensis: H 14
- Bacillus thuringiensis subsp. morrisoni: H 8a, 8b
- Bacillus thuringiensis subsp. thuringiensis: H 1

What its not

Bacillus anthracis

Anthrax

LD_{50} of two strains of *Bacillus anthracis* to albino Namru mice.

Strain	Route	Spores	Cells
D-380	IP	2.64	43.00
D-390	IP	6.73	8.20
D-380	Sub-Q	4.40	30.30
D-390	Sub-Q	5.75	3.03

Lamanna and Jones, J. Bacteriology, 1963.

Bt insecticides--What are they?

Essentially biologically produced chemicals. Product contains insecticidal crystal proteins, spores, lysed cell contents, and formulation ingredients.

How does it work?

Stomach Poison:
Crystals must be eaten

Each crystal contains several toxins

Toxins must dissolve to be activated

Crystal shape indicates activity

73 Holotype Crystal Toxins

1Aag	1Bae	1Cab	1Dab	1Eab	1Fab	1Gab
1Hab	1Ia-c	1Ja-c	1Ka	2Aac	3Aa	3Bab
3Ca	3Bab	3Ca	4Aa	4Ba	5Aac	5Ba
6Aa	6Ba	7Aab	8Aa	8Ba	8Ca	9Aa
9Ba	9Ca	9Da	9Ea	10Aa	11Aa	11Bab
12Aa	13Aa	14Aa	15Aa	16Aa	17Aa	18Aa
19Aa	20Aa	21Aa	22Aa	23Aa	24Aa	25Aa
26Aa	27Aa	28Aa	Cyt1Aa	Cyt1Ab	Cyt1Ba	Cyt2Aa
Cyt2Ba-b						

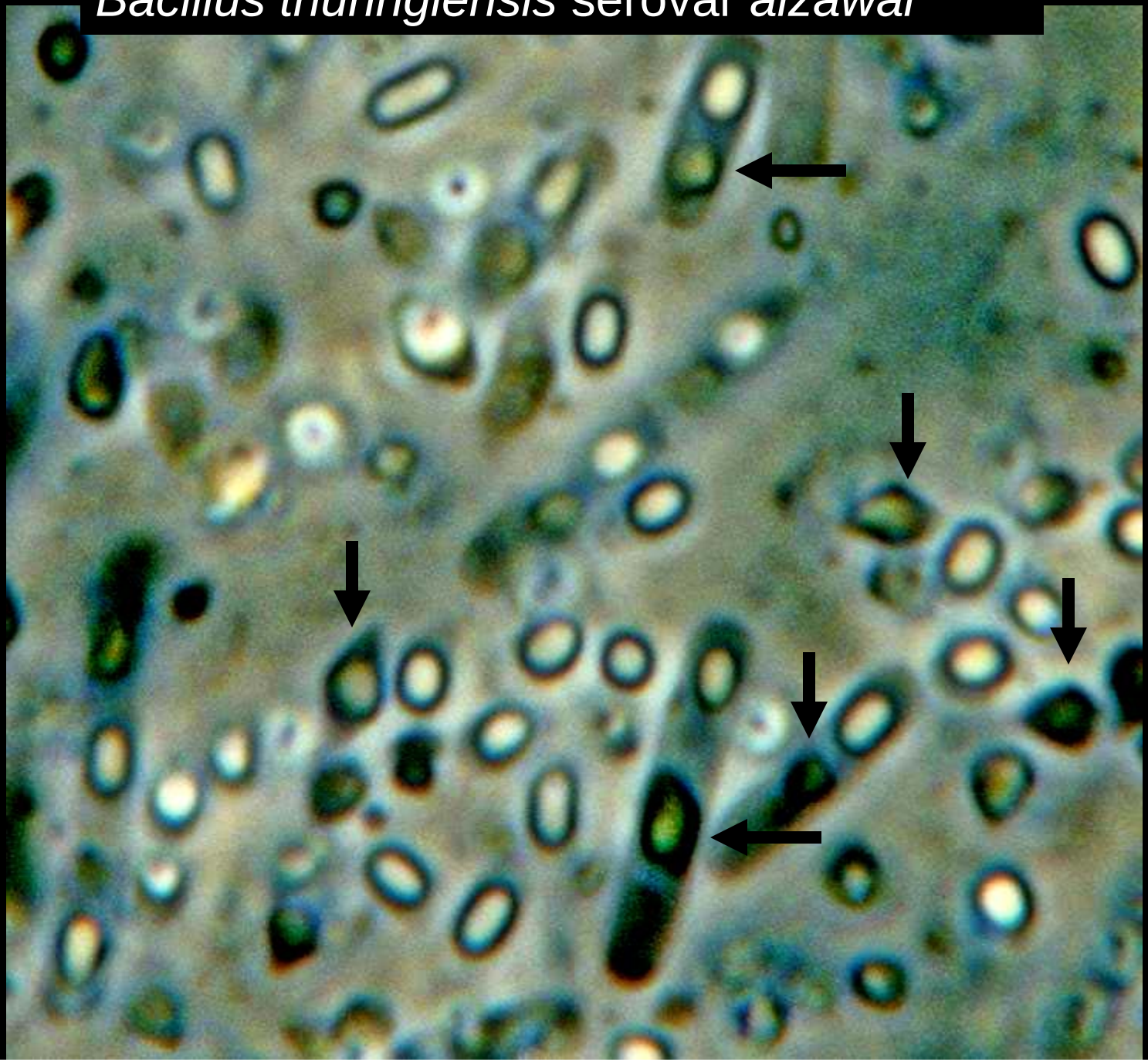
Toxins contained in Bt products

- **Dipel**, a Bt serovar *kurstaki* HD-1 product: **Cry1Aa, Cry1Ab, Cry1Ac, Cry2Aa, Cry2Ab**
- **XenTari**, a Bt serovar *aizawai* product:

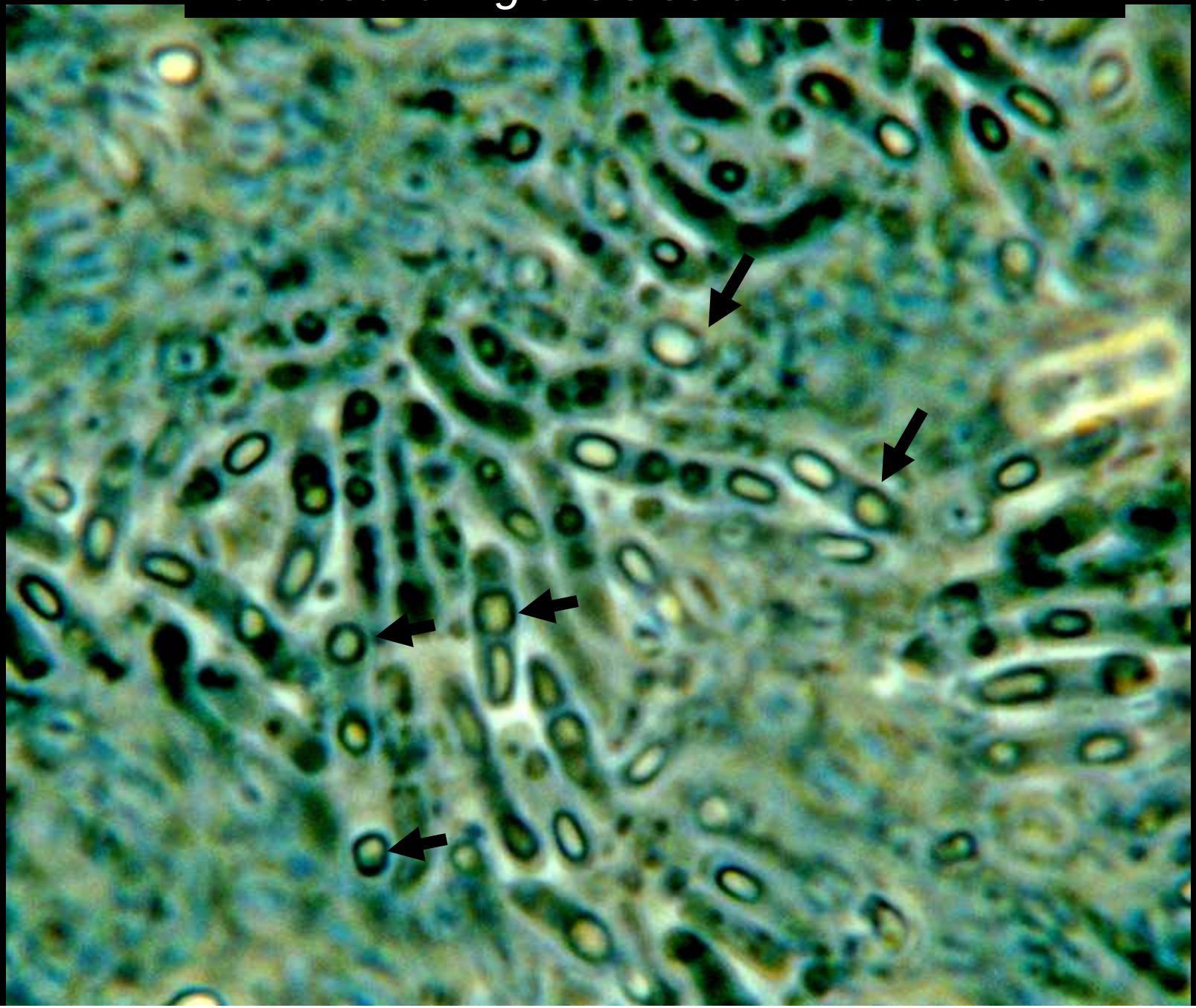
  Cry1Aa, Cry1Ab, Cry1Ca, Cry1Da, Cry2Ab

Bt Crystals

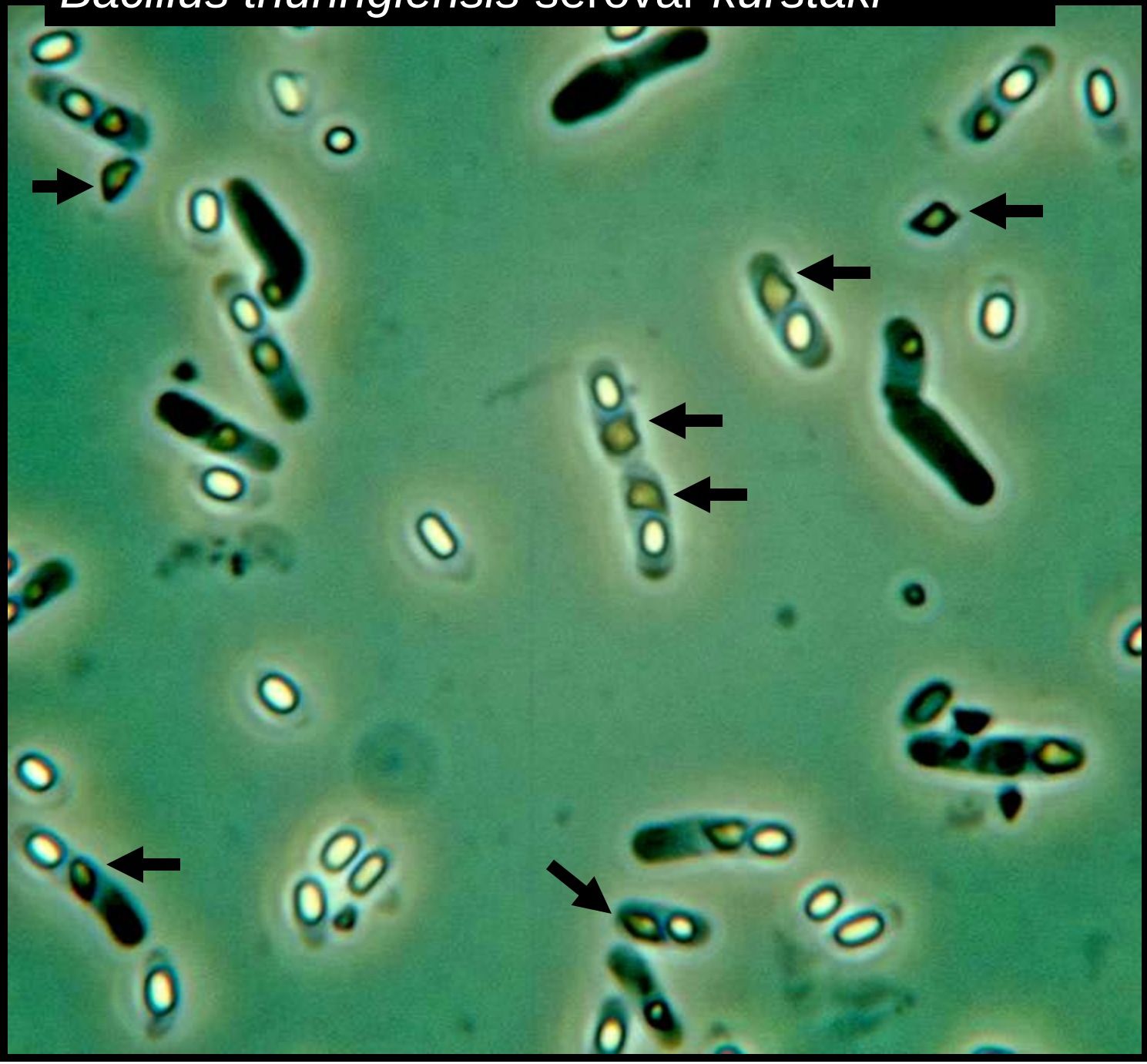
Bacillus thuringiensis serovar *aizawai*



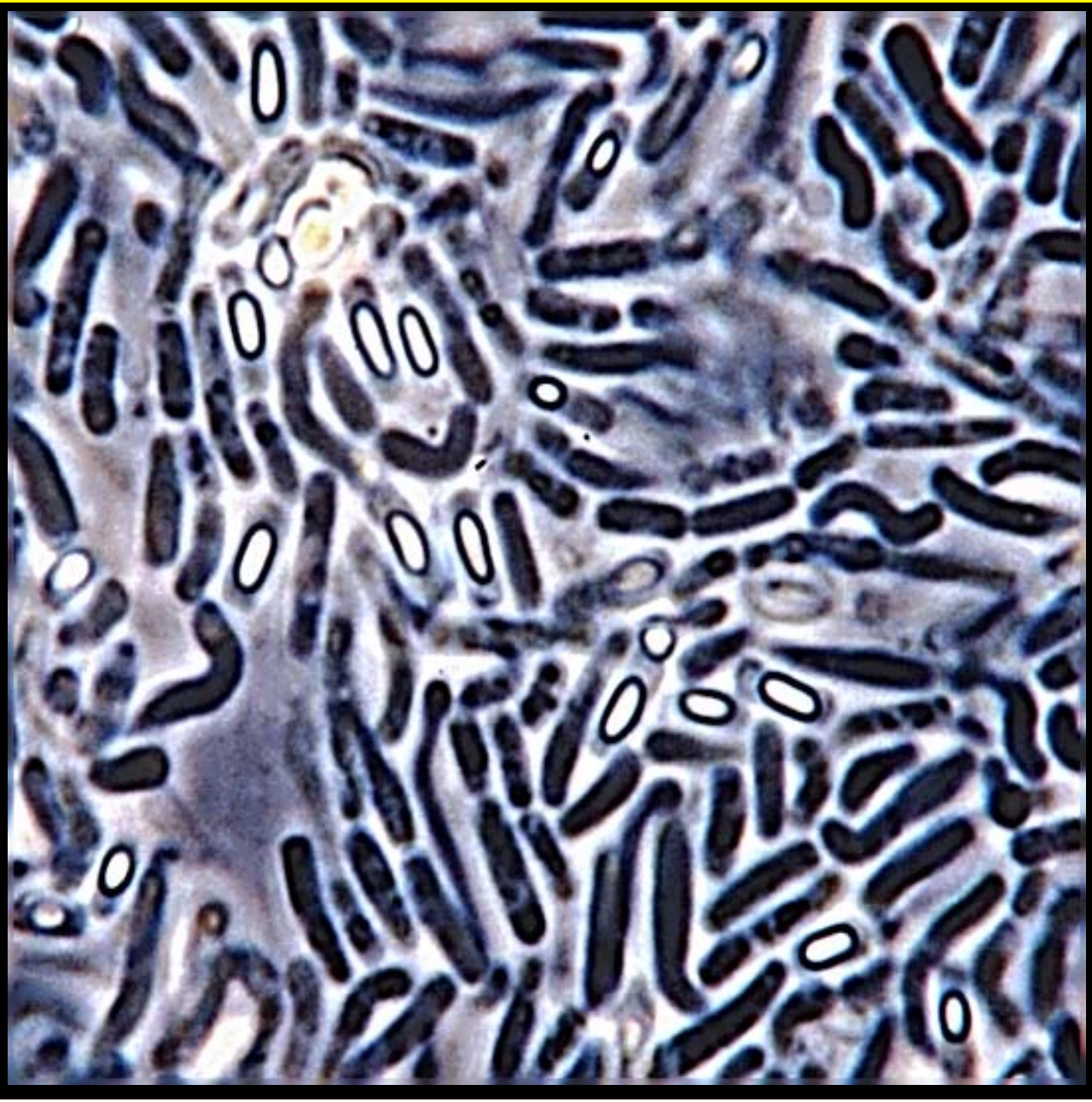
Bacillus thuringiensis serovar *israelensis*



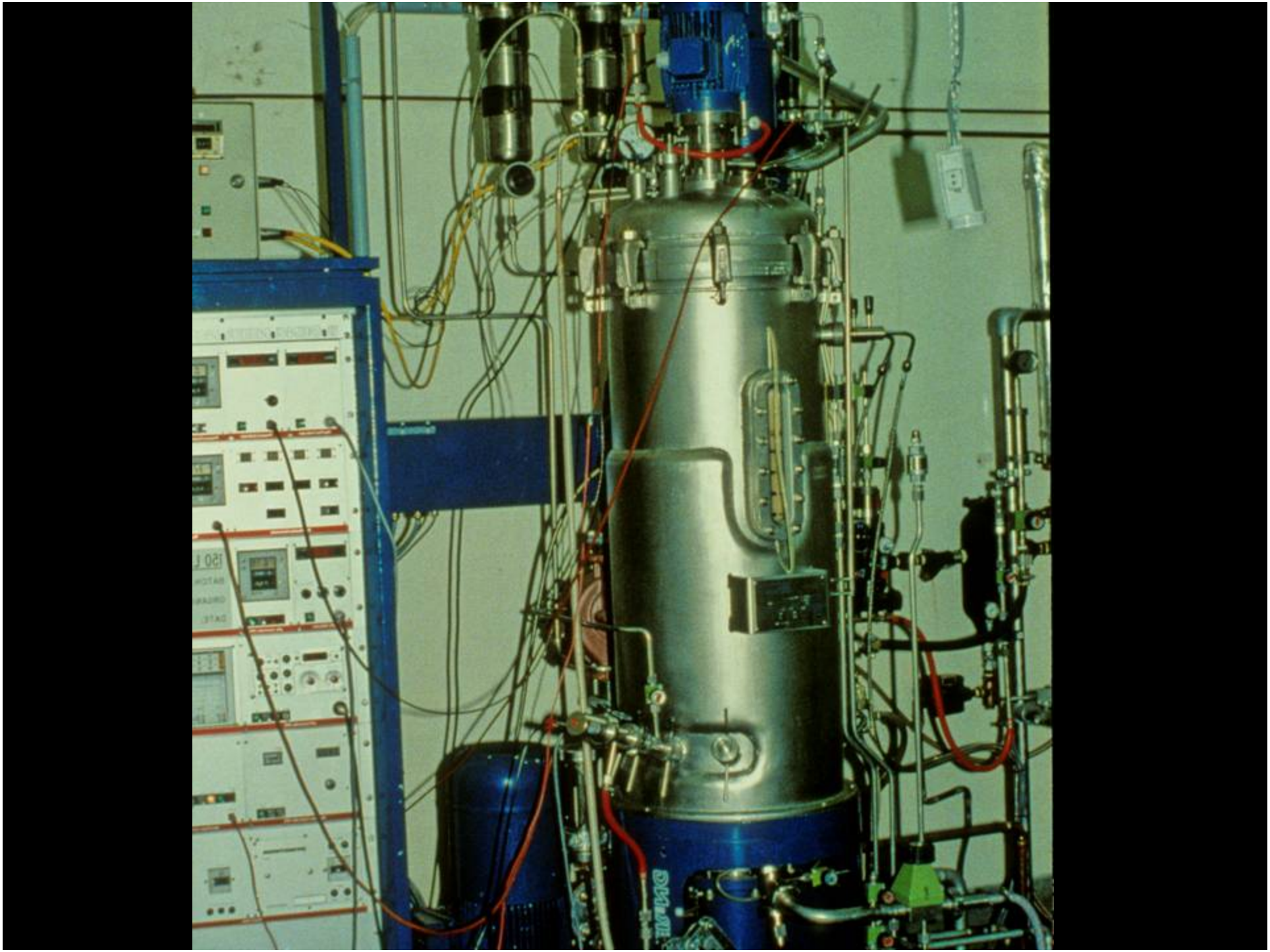
Bacillus thuringiensis serovar *kurstaki*



Bacillus cereus gastroenteritis outbreak



Production:
Stirred tank fermentation



- Production beer is purified and dried
- Product formulated as a techical powder
- Subsample assayed for potency
- Blended with formulation ingredients to improve flow, application and UV protection
- UV is a killer and crystal lasts a half day
- Crystals also degraded by soil bacteria. Spores may be degraded as well, do not compete well with other soil microorganisms, but some persist
- Naturally occurring Bt strains can be recovered almost anywhere

Some History

- Discovered in Japan in 1901 by Ishiwata
- Officially described by Berliner in 1915, isolated from Mediterranean flour moth in province of Thuringia in 1911
- Produced in France in late 1930's: WWII intervened
- US production of a subspecies *thuringiensis* in late 1950's
- Discovery of highly active subspecies *kurstaki*, HD-1, by Dulmage, 1960's, commercial production 1970s
- Discovery of mosquito, black fly active subspecies *israelensis* by Goldberg and Margalit, 1980's
- Discovery of beetle-active subspecies *morrisoni* by Krieg, 1980's
- **Genetically engineered cotton, corn, potatoes-ongoing**


SAFETY SAFETY SAFETY

Safety Studies Conducted on Bt

Ingestion- short term feeding with human volunteers

Long term feeding--2 years in rats, Cumulative dose in sheep as high as 10^{14} spores

Inhalation, including human volunteers**, Intranasal instillation**

Dermal exposure, Subcutaneous injection

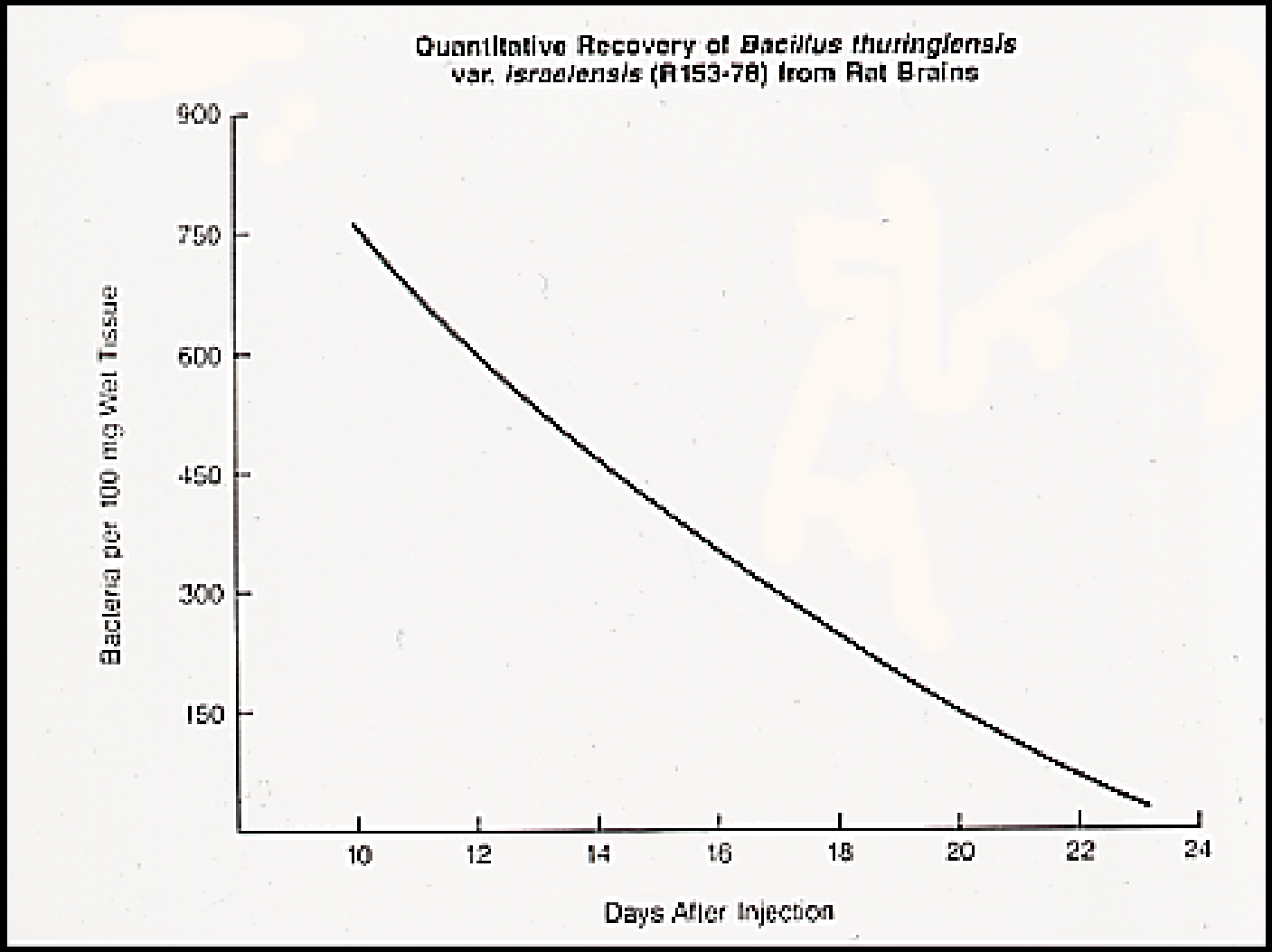
Intraperitoneal injection, including immune suppressed animals, Intracerebral injection, Intraocular injection

Dosage, Culture - Medium, Incubation Temp., and Route Matter!

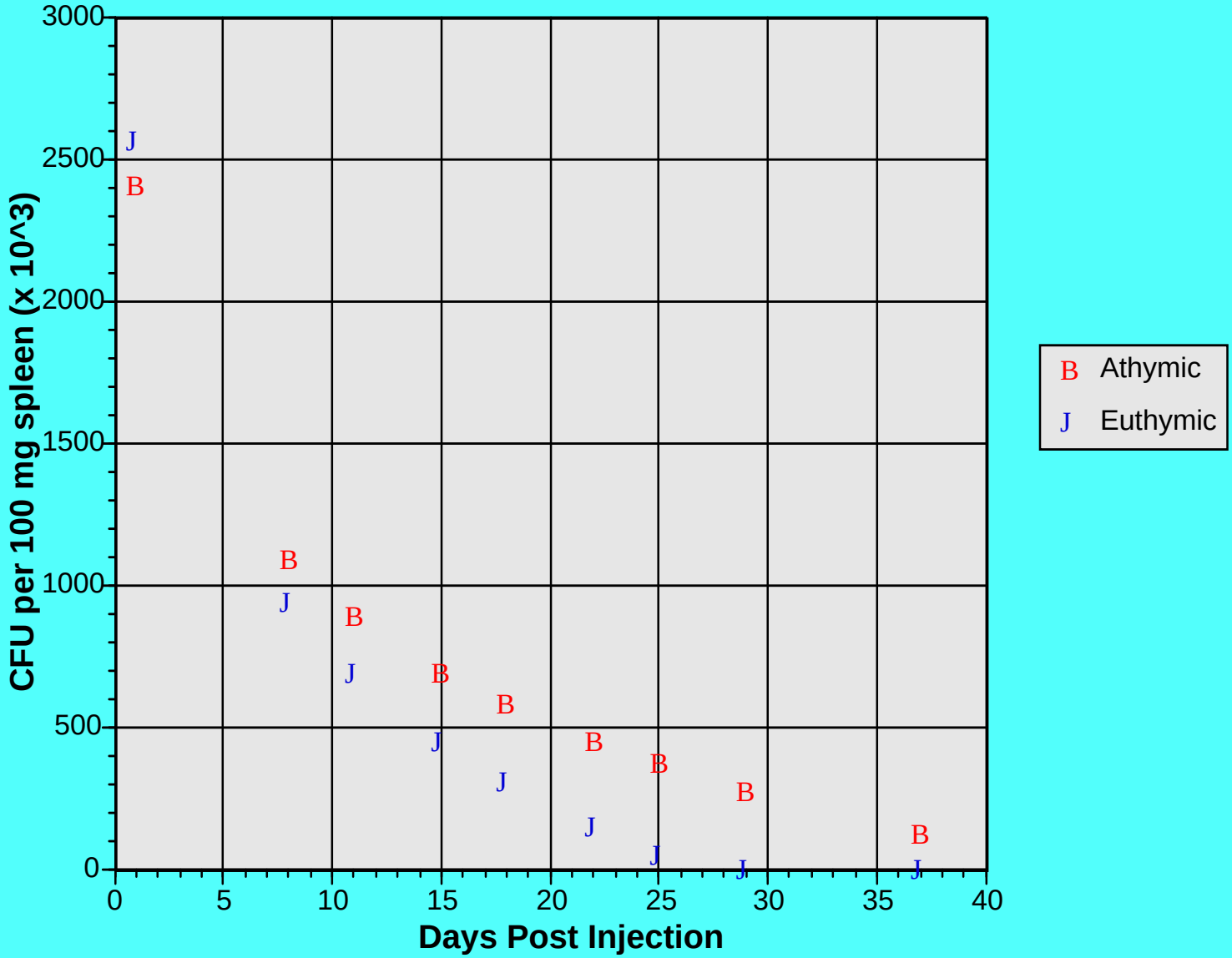
What is safe?

Mistaken belief that a "safe product" produces no mortality regardless of dose or route of administration

Clearance of Bt israelensis from rat brain. Dose was 10^5 CFU per rat, reported as recovery per 100 mg tissue



Clearance of *Bacillus thuringiensis* serovar *kurstaki* from athymic and euthymic mice, idealized clearance curve, 2 x 10^6 injected, (r^2=0.44, 0.51 respectively)



Major Issue

Is recovery of a microorganism from an animal or man synonymous with infection?


Persistence
Bti recovered 1-wk flushed eyes

Spray Campaigns

USA-Oregon 1985-1986

Green *et al.* 1990. Am. J. Public Health, 80.

Exposed: 80,000 people year 1
40,000 people year 2

Method: Monitor clinical laboratories, telephone complaint surveillance

Findings: 102 cultures examined, 55 positive for Bt; 51 ruled out as contaminants. 1 culture from eye splash. Bt not ruled in or out in remainder

CANADA- British Columbia 1992

Noble *et al.* 1992.

Exposed: 1,400,000 people

Sample size: 120 Ground Spray Workers; 1,085 physician patients; 744 lab cultures; 26,000+ hotline calls

Maximum exposure: 5.9×10^6 cfu m^3 air.

Findings: 104 spray workers were positive (nose swab); 126 physician cultures + BUT no link to illness. 42 pure hospital cultures-12 urine, 10 ocular

New Zealand: Auckland 1997

Anonymous: Public Health Protection Service

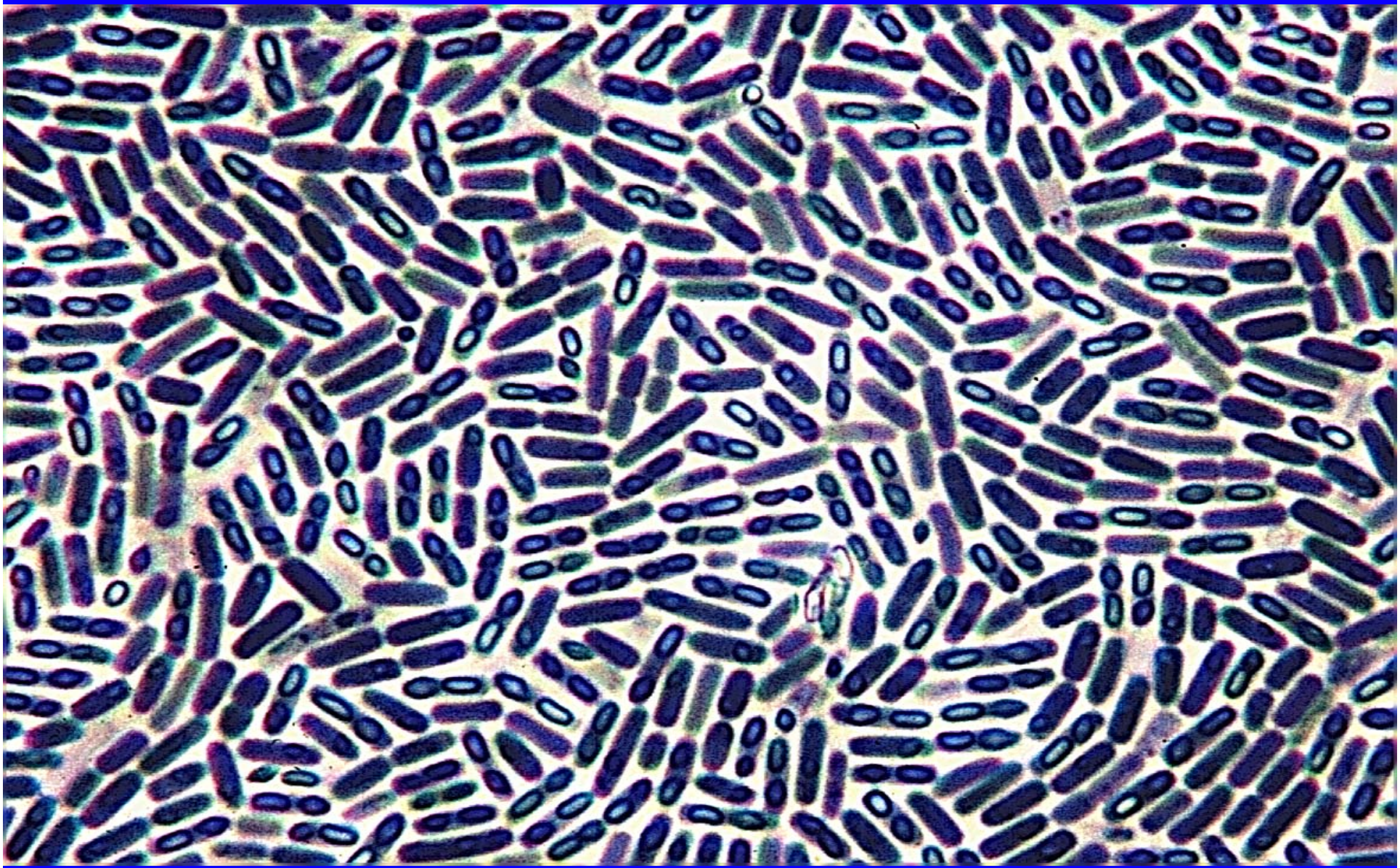
Exposed: 80,000 people

Outcome: No increased clinic attendance.

278 complainants-Eye, throat and skin irritation, headache. One Btk positive eyeswab, 1 Btk wound swab (also *Staphylococcus aureus*), 1 blood culture-skin contaminant.

Conclusion: No link to illness

Bacillus thuringiensis serovar *kurstaki*

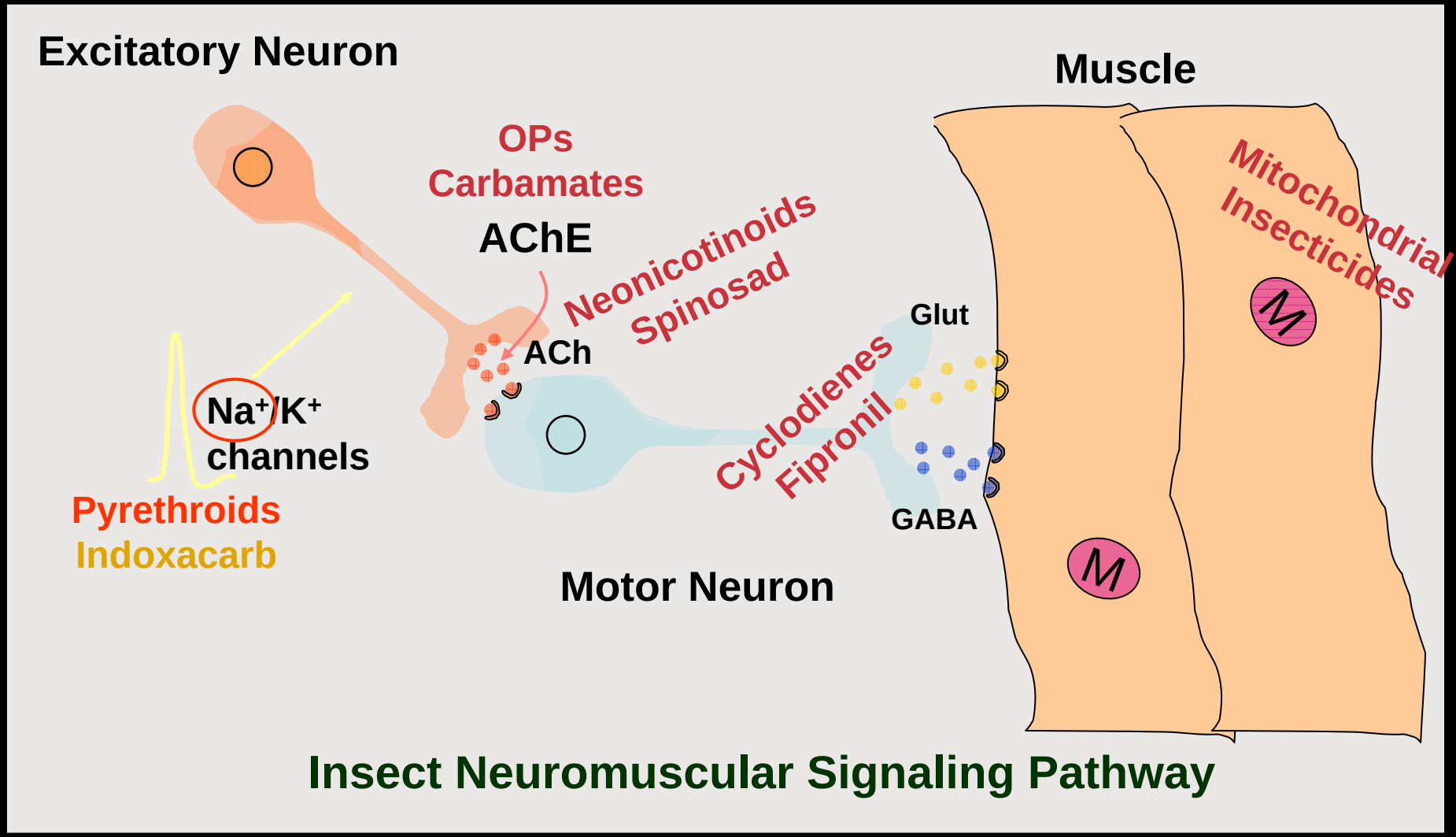


H.D. Burges. 1981. Microbial Control of Pests and Plant Diseases, 1970-1980.

“A ‘no risk’ situation does not exist, certainly not with chemical pesticides and even with biological agents one cannot absolutely prove a negative. Registration of a chemical is essentially a statement of usage in which the risks are acceptable. The same must apply to biological agents. ”

Borrowed from DuPont Crop Protection

Targets Below Reflect MoA for > 90% of Commercial Insecticides



Insect Neuromuscular Signaling Pathway