Bacillus thuringiensis: well armed bacteria to attack insects

Christina Nielsen-Leroux

Bacillus thuringiensis:
A well armed bacteria to attack insects

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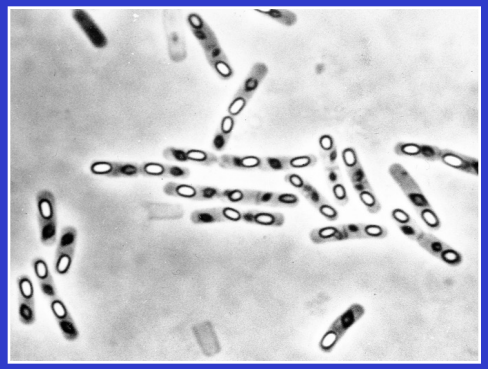


Bio-insecticide

Nuissance
Vector control = diseases
Malaria, Dengue...

Crop protection

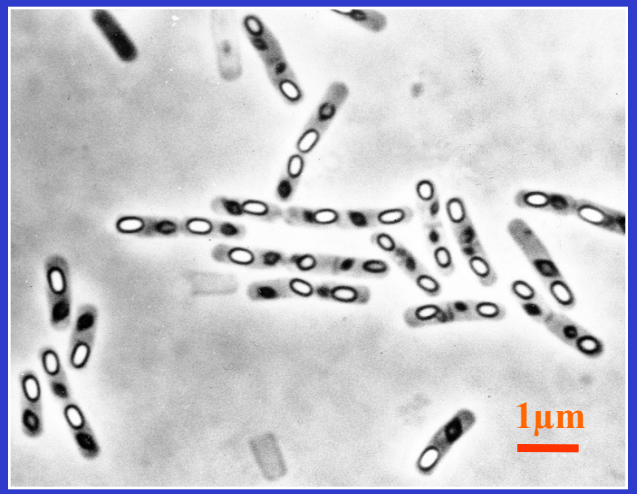
Bt-transgenic-plants

Cours AgroParisTech : AIP Santé, thème Science et Société : lutte contre le paludisme Février/Mars 2023

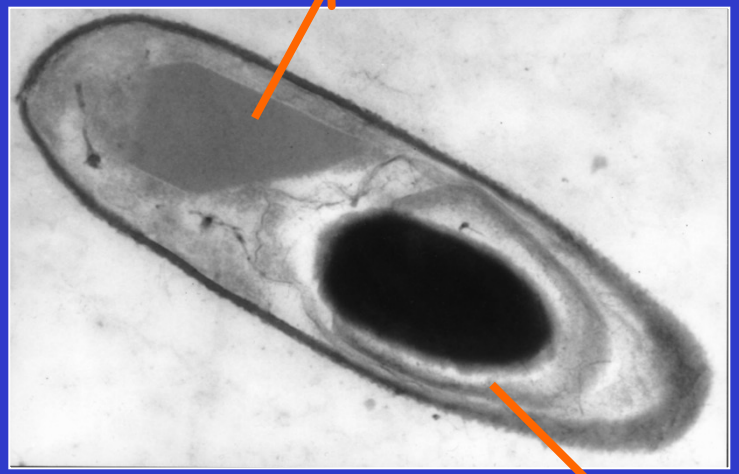
Bacillus thuringiensis is part of the *Bacillus cereus* group bacteria

Firmicutes, Bacillacae, Gram+, aerobic/ anaerobic, sporeforming, saprophyte, ubiquistic

Crystal / inclusion body = composed of cry toxin-proteins



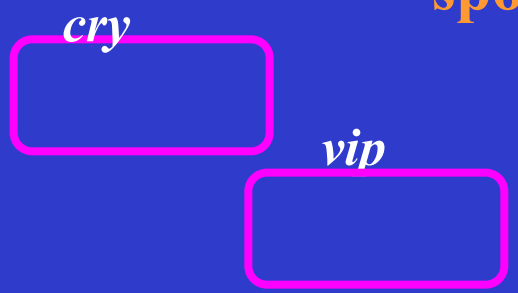
Light microscopy (Phase contrast)



Electronic transmission microscopy

spore

Specific Bt insecticidal toxin genes are plasmid-born



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Thousands *B.thuringiensis* strains isolated from around the world

- **Possessing one or more Cry toxins**

Classification of Cry toxins

Cry1Aa1... Cry74..

called three domain toxins

About 70 distinct groups

Also Cyt toxins

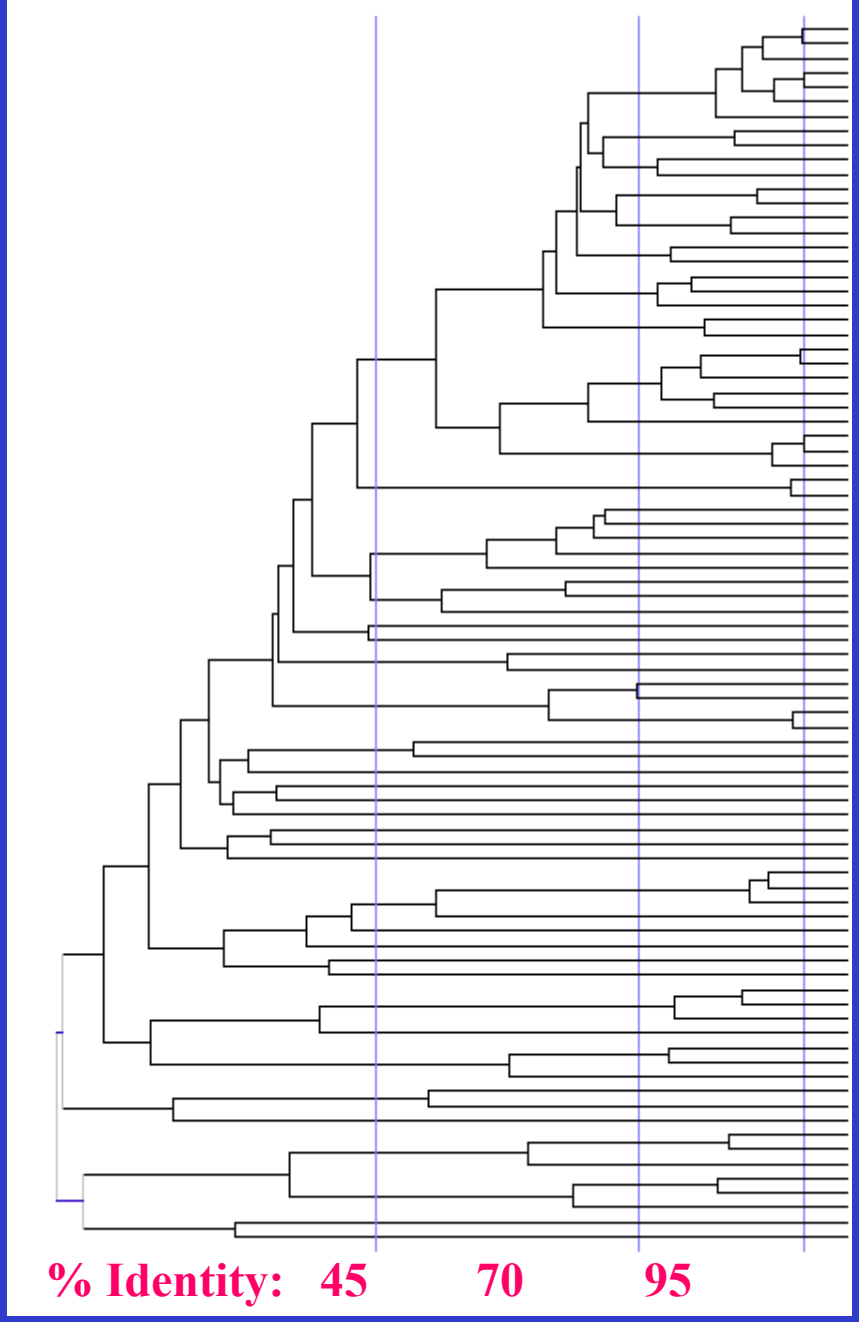
More than 600 differents sequenced genes

Specific targets :

- **Several orders of Insects**
- **Nematodes**
- **Mollusc**

Many toxins without yet identified targets

Bacterial pesticidal protein resource center :
www.bpprc.org



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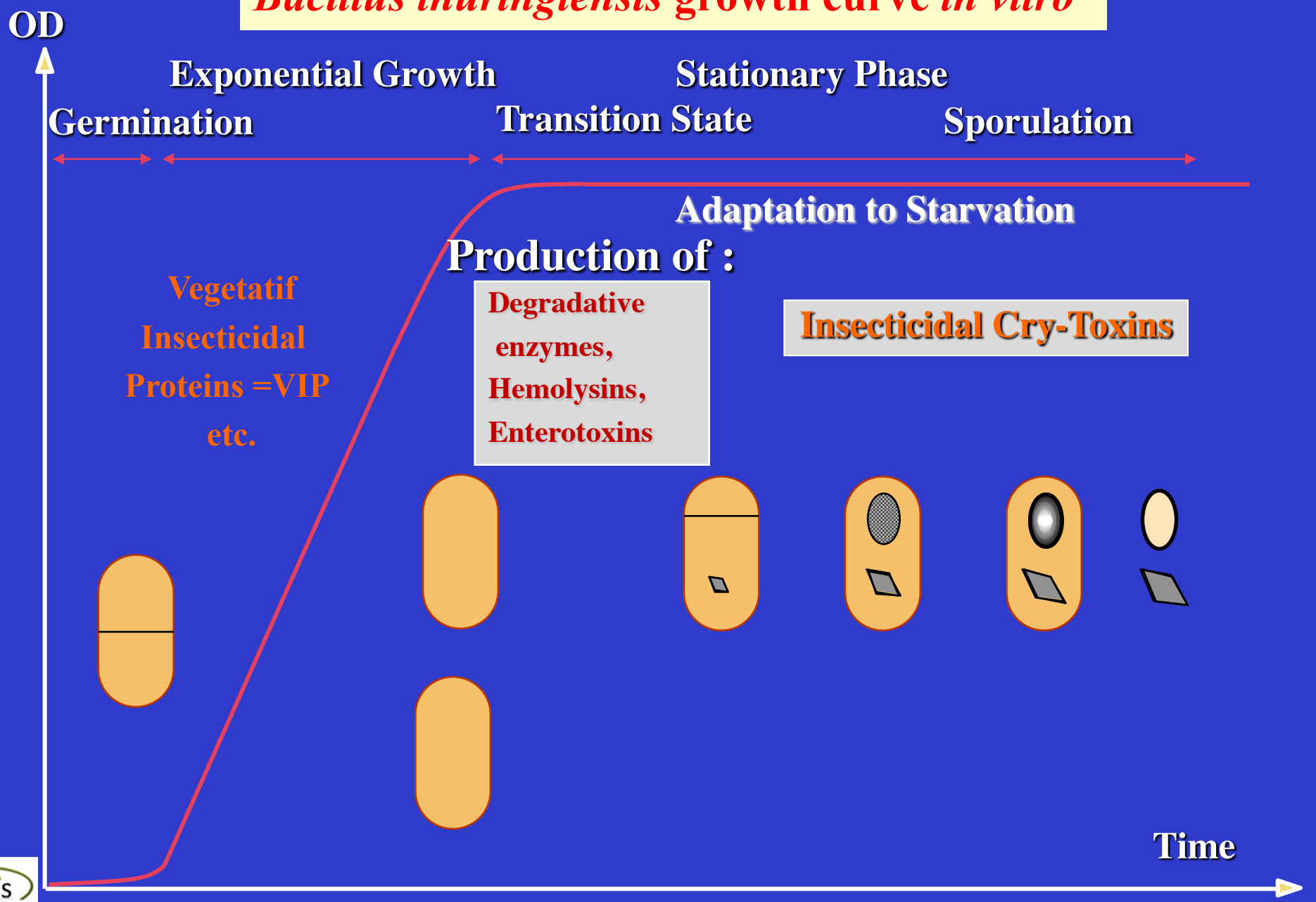
Bacillus thuringiensis growth curve *in vitro*



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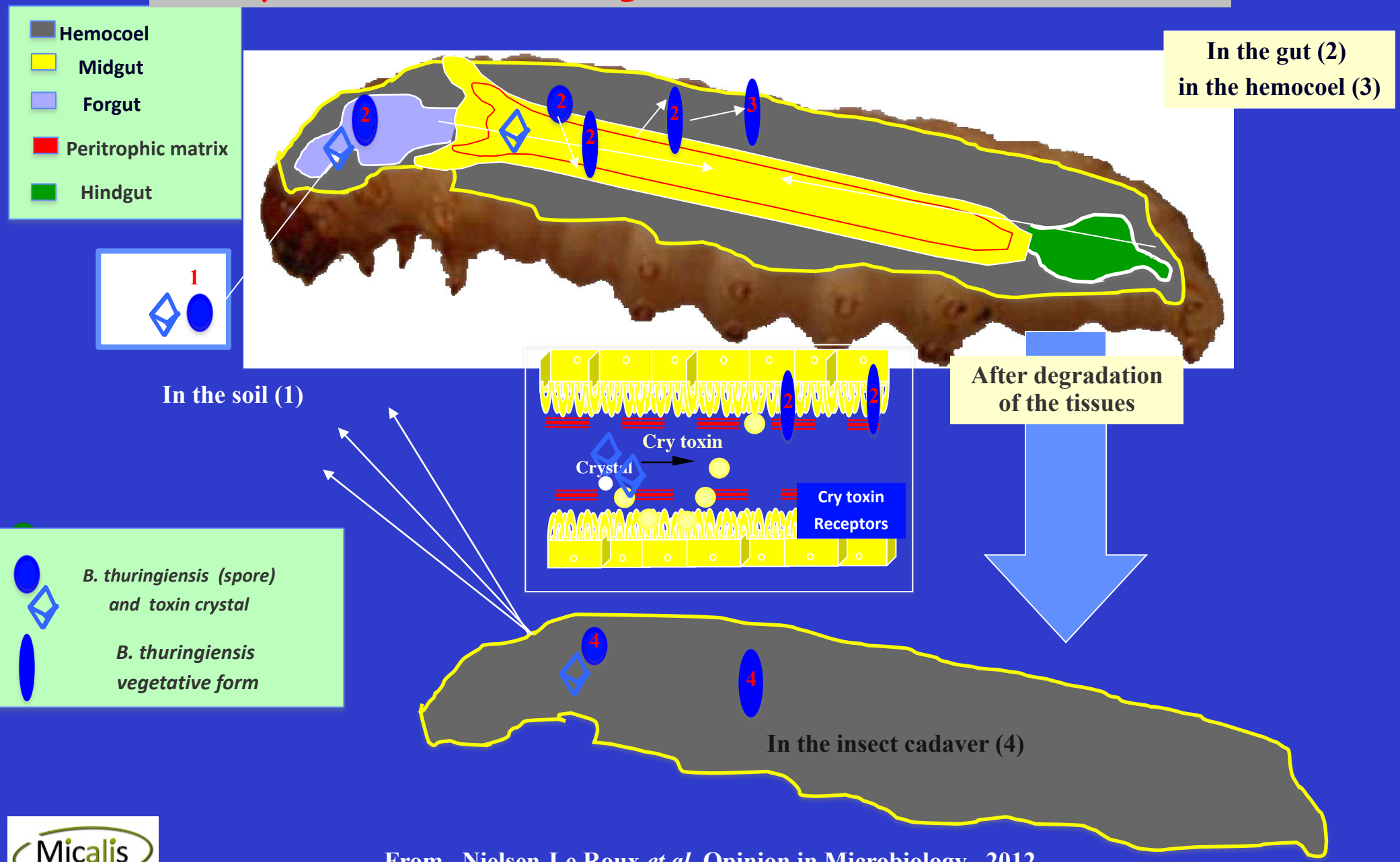
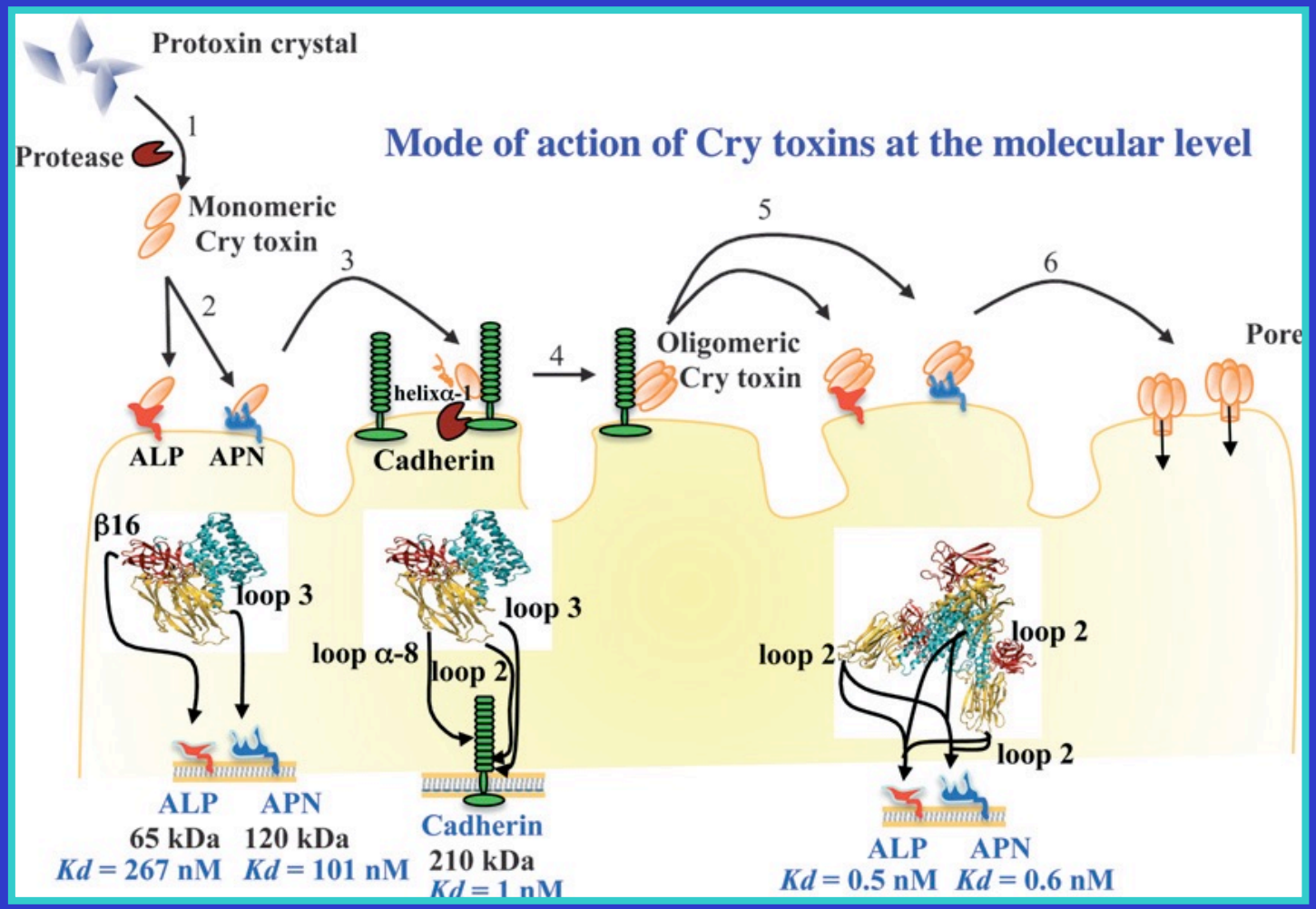
Lifecycle of *Bacillus thuringiensis* in the insect larvae (*in vivo*)



From, Nielsen-Le Roux *et al*. Opinion in Microbiology , 2012

Common mode of action steps of three domain Cry toxins



- Protease processing, receptor binding ...

(Soberon et al.., 2007, Pardo-Lopez et al., 2012)

Life cycle of main mosquito species

Adults

Eggs

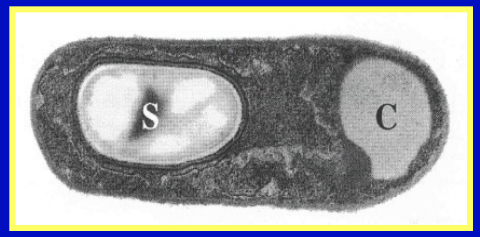
Pupae

Larvae

only stage
target for
Bt crystal toxins

B. thuringiensis sorovar.*israelensis* : Bti a bio-pesticide against dipterans

Bti



Oral larvicidal toxicity

specific target insects =

less side effects than chemical insecticides




Aedes *Simulium* *Culex* *Anopheles*

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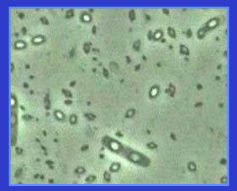
Bti Based larvicides

Adapted for conventional field treatment application

Fermentation →



Easy large scale production

↓

Biomass spores & Crystals

↓

Various formulations

https://publichealth.valentbiosciences.com

eg. Vectobac

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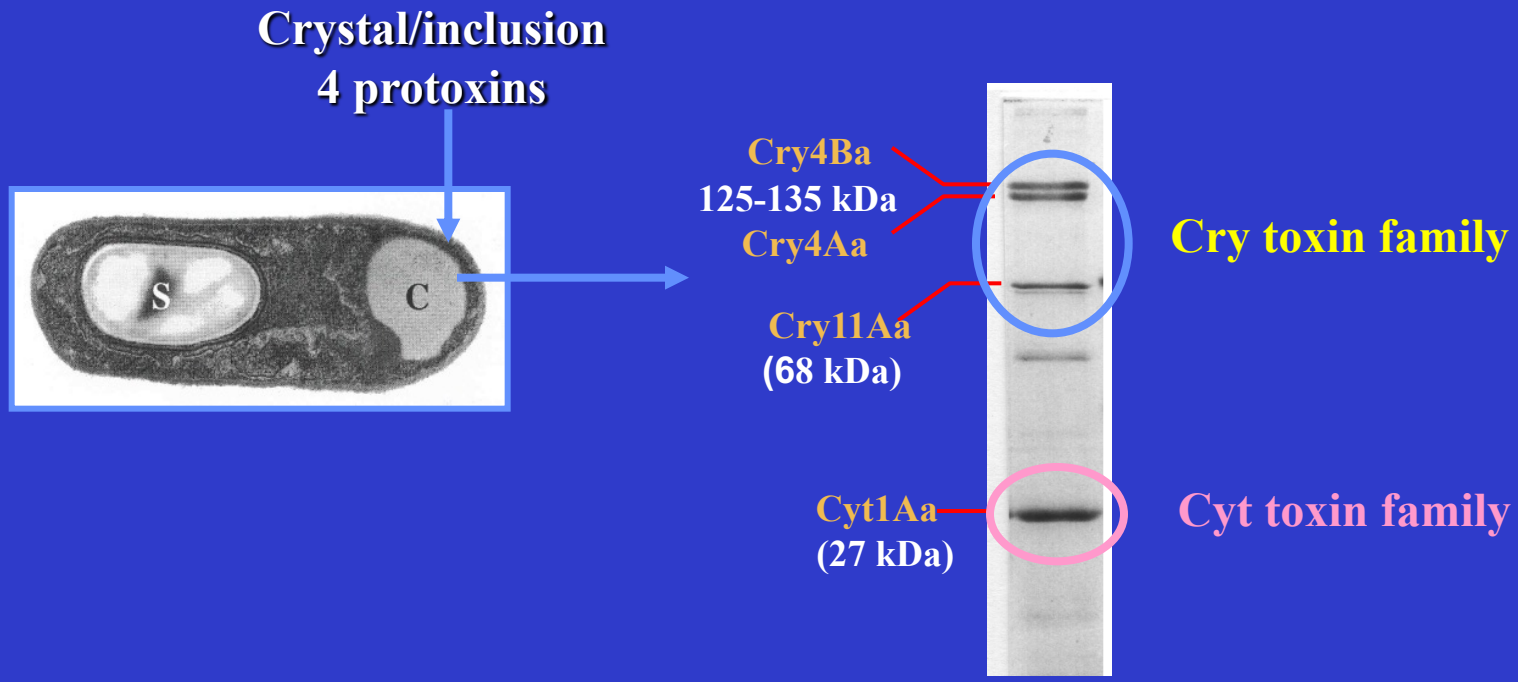
Insecticidal toxins of Bti and mode of action

Their potential against culicidae (moquitoes)

Specific targets = low environmental impact

Bti: crystal and insecticidal toxins

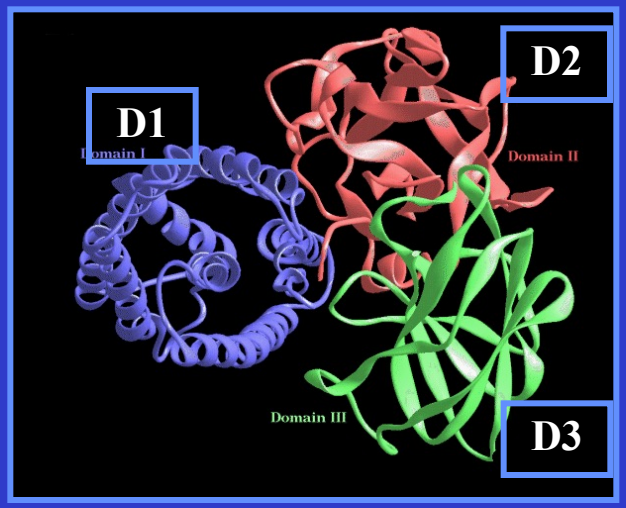


Activity spectrum: *Aedes, Simulium, Culex* & *Anopheles* = mosquistoe species = vectors of disease agents :

Plasmodium and arbo viruses like: Dengue, Chikungunya, Zika

Structure and function of Bti toxins

Cry: 3 domains



D2 and D3: Receptor binding

D1: Pore formation

Cyt: 1 domain α-β

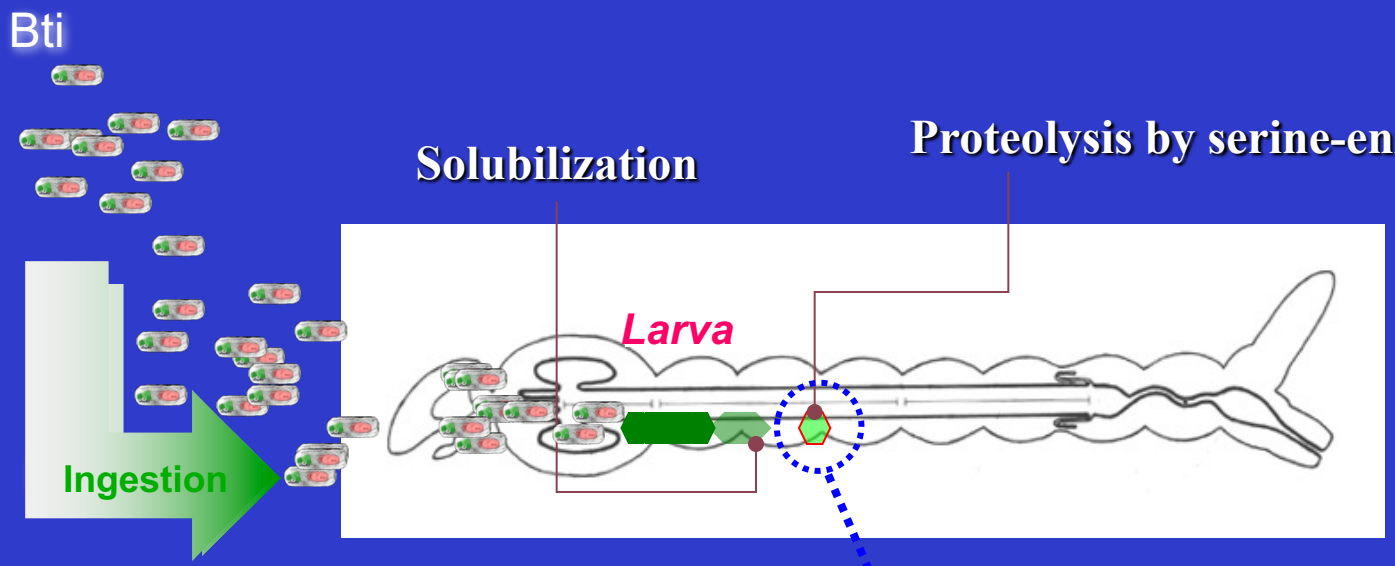
Cytolysis

Poreforming and /detergent effect

(Vachon et al., ., 2014)

(de Maagd et al., 2003)

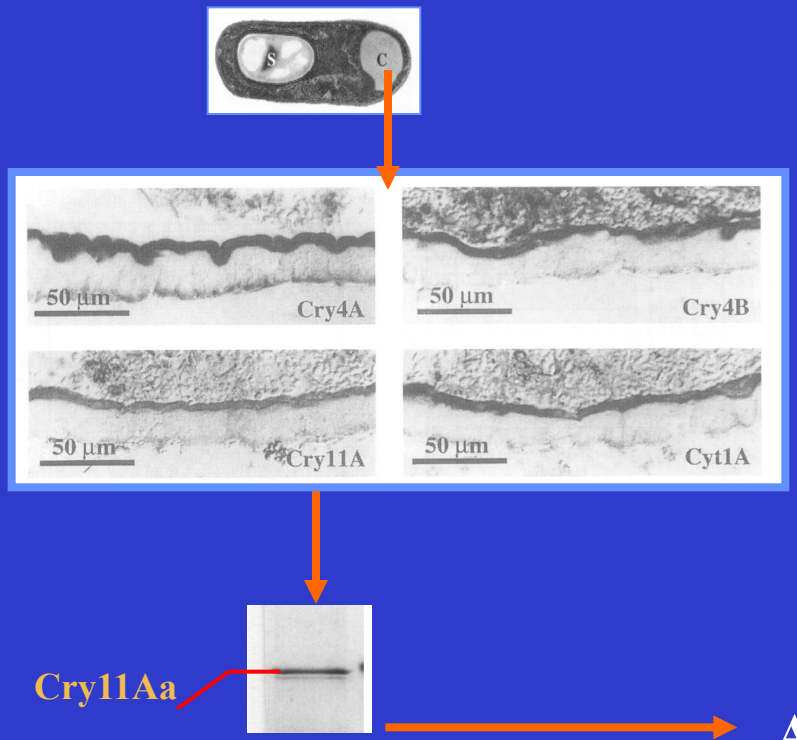
Mode of action of Bti crystal toxins

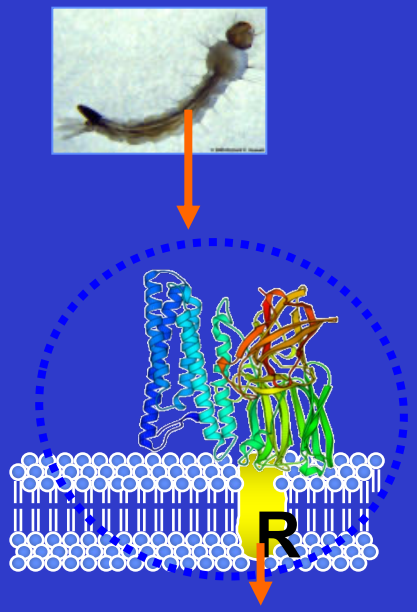


(Angsuthanasombat et al., 2006)

Cry11Aa receptor in *Ae. aegypti*

- Immuno toxin detection in the midgut of intoxicated larva





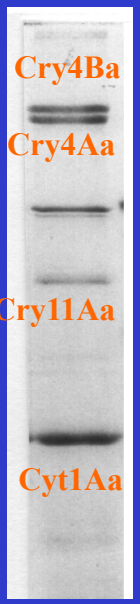
Alcaline phosphatase 65 kDa = receptor

Receptors are needed for intoxication

(Fernandez et al., 2006)

Synergy between the diffrent Bti toxins

(Crickmore et al., 1995; Poncet et al., 1997)



Toxicity (ng/ml) against *Ae. aegypti*

Compound	CL_{50}	Combination	CL_{50}
Bti native	10	Cry4A+CytA	75
Cry4A	1125	Cry11A+CytA	118
Cry4B	467	Cry4A+Cry11A	173
Cry11A	224	Cry4A+Cry4B+ +CytA	77
CytA	1209		

CL_{50}=concentration that kills 50 % of the exposed larvae)

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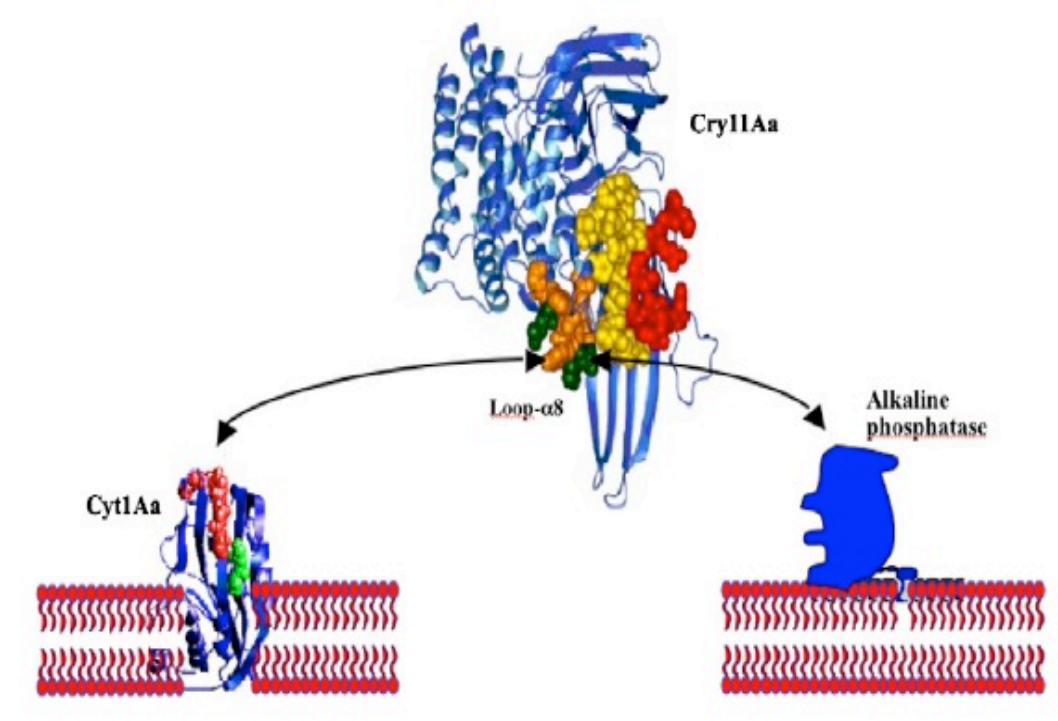
Synergy between Cry11Aa and Cyt 1Aa



Cry11Aa binds to the membrane receptor

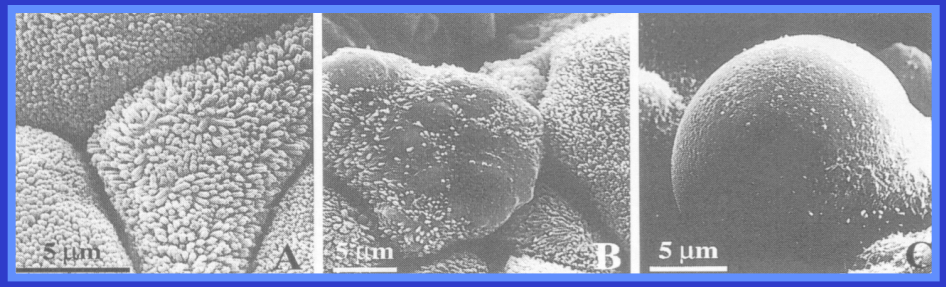
Cry11Aa binds also to Cyt1Aa as a second receptor

(Pérez et al., 2005; Soberón et al., 2009)

Effects of Bti crystal toxins on intestinal cells of Aedes aegypti

Microvilli of midgut cells

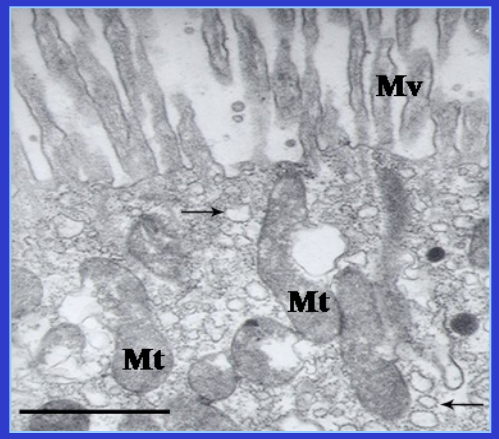
Scanning Electronic microscopy

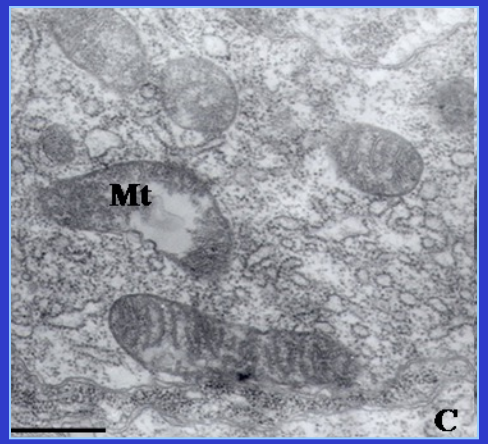


0 min 30 min 1 hour

Midgutcells

Transmission electronic microscopy





Charles *et al.*, 1988

Bti is very efficient in mousquito control
is there a risk for development of resistance in treated populations ?

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Link between Mode of action and Resistance mechanisms

- Modification of receptor binding sites
  - -Risk of selection for resistance/ lower affinity
- Modification of toxin processing
  - -proteolysis of protoxins
- Detoxification enzymes
  - -Processes often seen for synthetic insecticides
  - - Mono-oxidases, transferases, esterases

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Selection of resistance under laboratory conditions

Species	Generation	Resistance levels: Bti	Cry4A	Cry4B	Cry11A	Cyt1A	
C. pipiens	28	3.2	___	___	913	___	Georghiou & Wirth 1997
	35	4.4	___	___	16	2	Wirth et al. 2010
	20	3.0	___	___	___	___	Mittal et al. 2005
	20	2.8	___	___	___	___	Saleh et al. 2003
Ae. aegypti	15	1.1	___	___	___	___	Goldman et al. 1986
	15	1.1	___	___	___	___	Goldman et al. 1986
	15	2.0	___	___	___	___	Goldman et al. 1986
	18	3.4	14	6	30	3	Bonin et al. 2010
	18	3.0	60	4	7	4	Paris et al. 2010
	22	___	35	11	3	___	Paris et al. 2011
	54	___	___	___	13	___	Cadavid-Restrepo et al. 2012

In the laboratory very low resistance to the full Bti toxin mix but resistance is posible to the individual toxins

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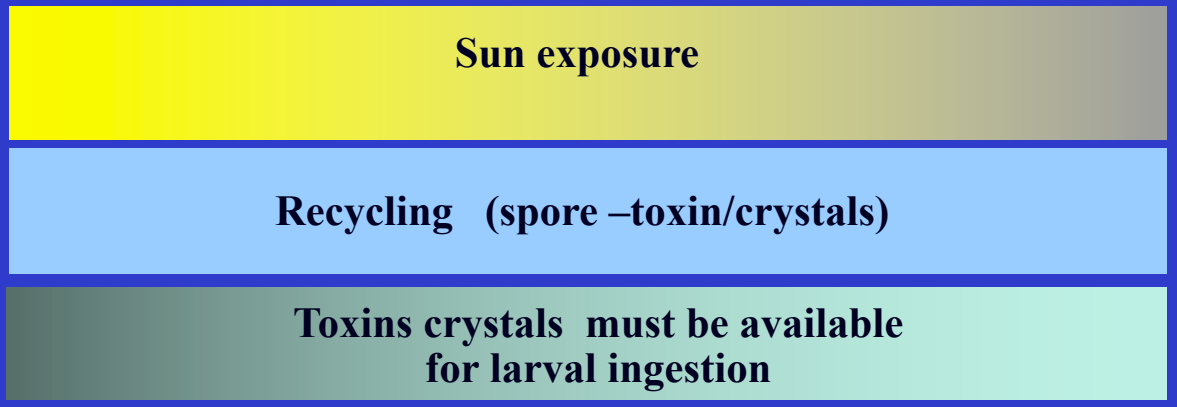
Exemples of Bti field applications

Persistance of Bti in the field

Tablet comercial	90-112 days	Mulla et al., 2004
Tablet comercial	54-166 days	Benjamim et al., 2005
Tablet experimental	84 days	Armengol et al., 2006
Commercial formulation	35 days	Lee & Zairi, 2006



Persistance is larval breedingsite and formulation dependent

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Control of *Simulium (Blackfly)* - againt Onchocerciasis (River blindness) with Bti In west Africa (OCP program)

- Actors : OCP/WHO
- Period: 1982- 2002
- Target: *Simulium damnosum* complex
- Region: 11 countries /1.3 Mill Km^2/25 .0000 hab.
- Results:
  - Control of **Onchocerciasis**
  - Reimplemenation of inhabitants
  - Strong socio- economic impact

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Control of *Ae. vexans* in the Rhine valley, Germany (KABS)

- Actor: KABS
- Period: 1983- still ongoing
- Bti is "gamma irradiated" = no viable spores
- Target: *Aedes vexans*
- Treated surface: 600 km2
- Results:
  - Good efficacy, optimized formulations
  - Ecological survey,
  - Positive impact on tourism

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Control of *Simulium* sp. in Brazil

- Actor: Gouvernment (Health secretary)
- Period: 1982- still on going
- Target: *Simulium pertinax*
- Region:

Serra Gaúcha S: 43,000 km^2 /3 milhões hab.

Litoral Norte de SP: 872 km^2

- Results:
  - Efficacity
  - positive Impact population and tourism

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Use of Bti in France

- Actor: EID (Entente interdepartemental de démoustication)
- Period: 1990- still ongoing
- Target: *Aedes, Culex spp*
- Region:
  - **Rhônes-Alpes : www.eid-rhonealpes.com**
  - **Mediteranean** http://www.eid-med.org
  - **Atlantic coast www.eidatlantique.eu**
- Results:
  - Decrease in nuisance
  - Positive Impact on population and tourism

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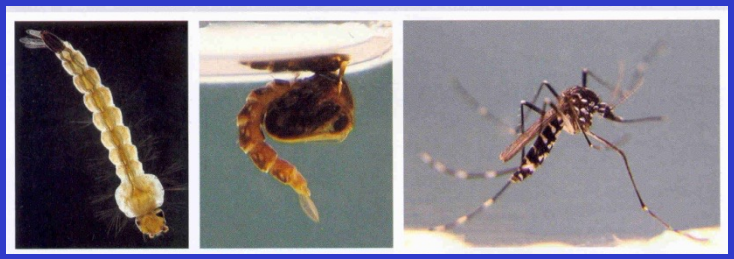
Integrated control of *Aedes* in Brazil

Excellent larvicidal activity
field efficacy
Possibility to be used in integrated control



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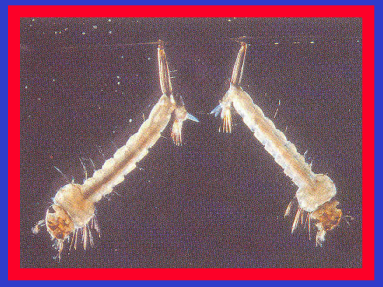
Life cycle of main mosquito species

Adults

Pupae



Larvae

Eggs

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Control of *Aedes*: integrated control measures are needed

Monitoring

Elimination of egg laying areas

Capture of adults mousquitoes

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Use of Bti in Brazil to control Dengue disease

Aedes
Dengue

1981 → 1986 → 1996 → 2002 → ...

2000-2002: Detecion of resistance against Organophosphate insectides (OP)

Application of Bti againt OP resistant populations

Bti as a possible alternative to Temephos

- Resistance to Temephos is related to detoxication enzymes
- Is there a possibility of cross resistance between Bti and Temephos ?

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Bti Susceptibility of *Aedes* populations (strains)

with moderate resistance to Temephos

Strain	exposition	Temephos RR	RR Bti
Rock	Non	1	1.0
F. Noronha	Bti	2.4	1.6
Recife	T-Bti	7.1	1.8
Bacabal	T-Bti-IGR	6.6	1.8
Macapa	T-Bti-IGR	11.0	1.5
J.Pessoa	T-Bti-IGR	11.7	1.4

T= Temephos, IGR = inhibition of growth regulator

RR= resistancde level ; 1 = no resistance

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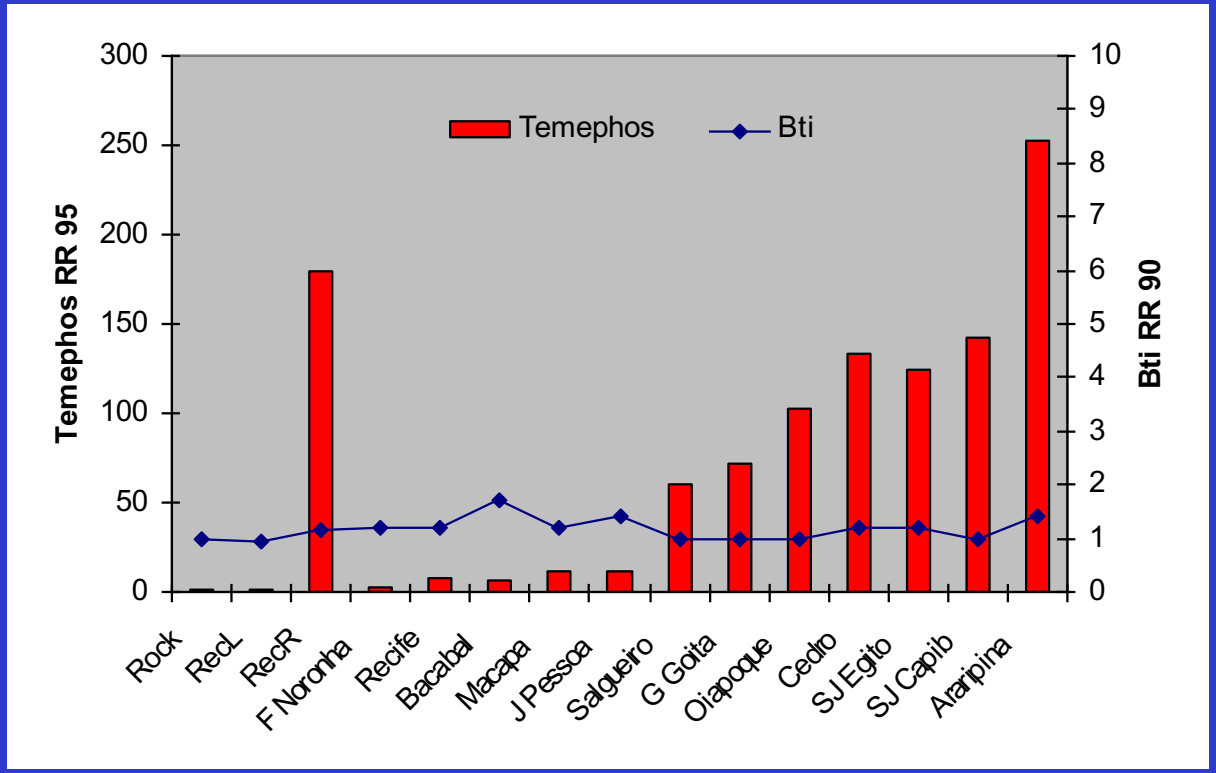
Resistance ratio (RR): Temephos and Bti



Temephos RR: 2 to 253 fold **Bti RR: 1 to 2 fold**

No cross resistance to Bti

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Conclusion related to Bti

- Bti very low risk for resistance development (several toxins and different host receptors, a complex mode of action)
- No cross resistance between Bti and the Organophosphate insecticide Temephos
- Bti can be used to control of *Aedes* and other mosquitoes like *Anopheles* to decrease malaria

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Are Bt strains really without risk for non targets ?

- Recommendet for use in drinking water (WHO, 1999, 2012) !
- https://www.efsa.europa.eu/fr/efsajournal/pub/4524 2016

  Risk of Bt based larvicides ?

Issues : Not only Cry toxins in Bt Products

What about the spores / bacteria ?

Bt is part of the *B. cereus* group !

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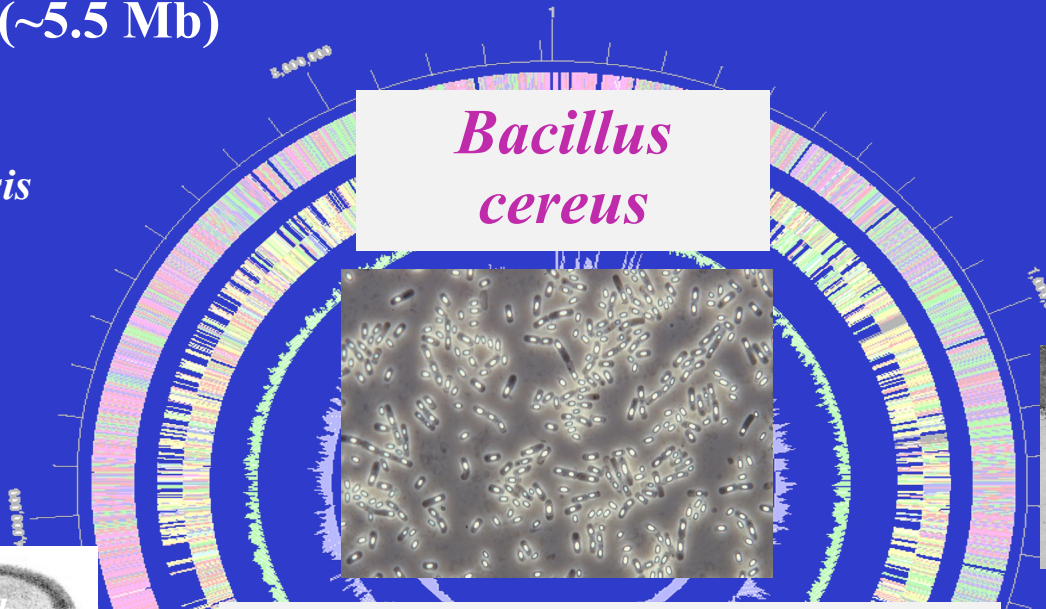
Bacillus cereus group

Bacilli, Gram positif, spore forming & ubiquistic

Common gene pool (~5.5 Mb)



Bacillus cereus

Bacillus cereus

Opportunistic pathogen

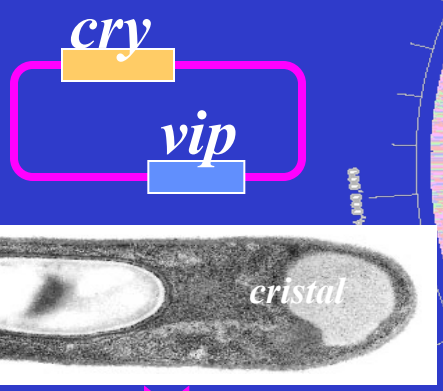
Human

Animal

- **Gastro intestinal diseases**
- **Local and systemic infection**

Bacillus thuringiensis

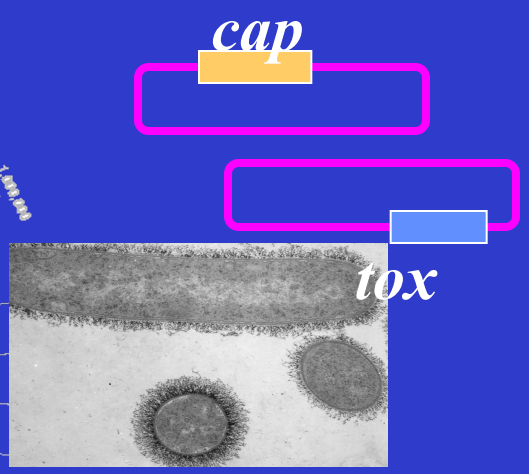
Specific genes



Entomopathogen

Crystal = specific ''Insecticide"

Bacillus anthracis



Animal & human pathogen

'Antrax disease"

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Are Bt strains really without risk for non targets ?

- Recommended for use in drinking water (OMS, 1999, 2012, but not at present) !
- https://www.efsa.europa.eu/fr/efsajournal/pub/4524 2016

risk of Bt based larvicides ?

Issues : Not only - Cry toxins in Bt Product

What about the spore/ bacteria

Member of the *B. cereus* group !

Need for « OGM » Bt strain ?

Without spores =

MosKO project

in the Micalis GME team

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More than 60 years of use in rivers, forest without problems !

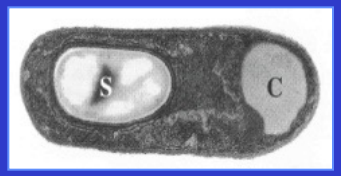
Chemicals will be prohibited = expect increased use of bio-pesticides

Objectifs of Moskill/ MosKO

https://satt-paris-saclay.fr/en/technological-projects-portfolio/mosko/

A new safer and more efficient mosquitocidal larvicide

Today



Bt *serovar israelensis* (Bti), the only mosquitocidal larvicide autorized in France
Spores are spread into the environment

The Bti toxins are very efficient

But :

- Need to increase the bio-availability of the Cry toxin's due to UV degradation, adsorbance to soil
- The spores are spead into the environment = water= potential risk in food contamination

Objectifs/ project ongoing

MosKO

- **Absence of bacterial spores**
- **Bio-larvicidal toxins**
  - ✓ **Several toxins= increased efficacity/more targets**
  - ✓ **Protection against UV**
  - ✓ **Increased bio-availability**

MosKill/MoskO

Encapsulated crystals
NO spores

« Muito obrigada » to my freind and colleague

Maria Helena Neves Lobo Silva Filha
FIOCRUZ -PE, Brésil

Merci pour votre attention !