PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

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Bacillus thuringiensis strains and its toxicity

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Table of contents

Introduction

As hazards of conventional broad acting pesticides are documented, researchers, policymakers and growers look for pesticides that are toxic only to the target pest, have no impact on other such as beneficial species, and have fewer environmental effects. *Bacillus thuringiensis* (Bt) insecticides results from this research and is a typical example as one of the components in an IPM approach. Among the various Bt strains insecticidal activity is specific. However, care is warranted in its use because
depending on the Bt strain and production method e.g. non-specific heat-stable exotoxines (sometimes,structurally similar to a nucleotide) can be produced which can have negative side effects. So far detectable levels of ß-exotoxin are not allowed in *B. thuringiensis* products in Western Europe and North America. Out of concern for mammalian toxicity, the World Health Organization (1999) addressed non-specific thuringiensin from different subspecies of Bt to promote human health, and regulated Bt strains that produced thuringiensin.

Description of *Bacillus thuringiensis* (Bt)

Introduction

Bacillus thuringiensis is a facultative anaerobic gram-positive bacterium. It is rod-shaped and spore-forming.
B. thuringiensis is closely related to *Bacillus cereus*, another species of the genus *Bacillus*. The difference between the two species is the ability of *B. thuringiensis* to produce a protein crystal during sporulation.
B. thuringiensis occurs naturally in soils, in water and on leaf surfaces in many parts of the world.
In 1901 a Japanese researcher discovered the bacterium in diseased silkworms. Bt has been used as an insecticide since 1938 but it was not successfully commercialized until the1950's, at first as a biopesticide for the control of lepidopteran pests (caterpillars). In the late 1970's many more Bt-based insecticides were developed against a lot of other pest insects, such as mosquitoes, flies, beetles. One of the advantages of these microbial control agents is that they can be highly selective, infecting or killing a very narrow range of target insects. So, beneficial insects will not be killed. The diversity in insecticidal uses of the different Bt products is caused by the diversity in **genetic properties** of the many subspecies, serovars and strains within these subspecies of *Bacillus thuringiensis*. Several systems of classification of Bt are developed.

Diversity in possible toxins of Bt

1. δ-endotoxin (delta endotoxin), a group of protein crystals

The main insecticidal effect of Bt is based on the crystal that is produced in the bacterium during sporulation. This crystal consists of a protein, the **δ-endotoxin**.
When this δ-endotoxin is ingested by an susceptible insect larvae, it reacts with the cells of the gut, causing perforations. The mixing of haemolymph and gut contents creates favorable conditions for Bt spore germination and vegetative proliferation, which may result in septicemia (blood poisoning, haemolymph). The insect will stop feeding and will die of starvation, within hours to several days.

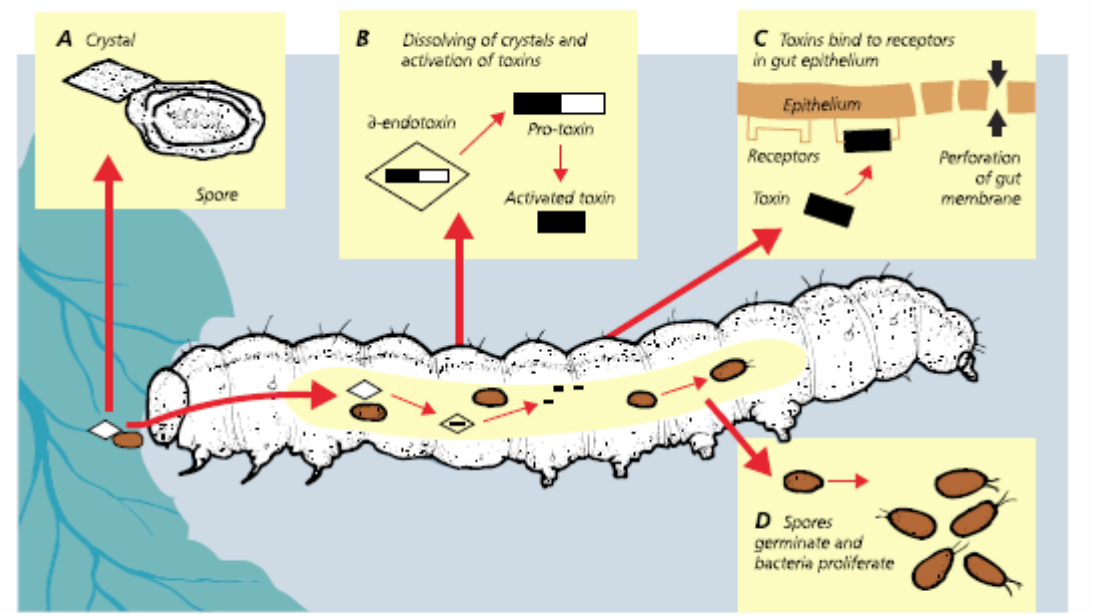


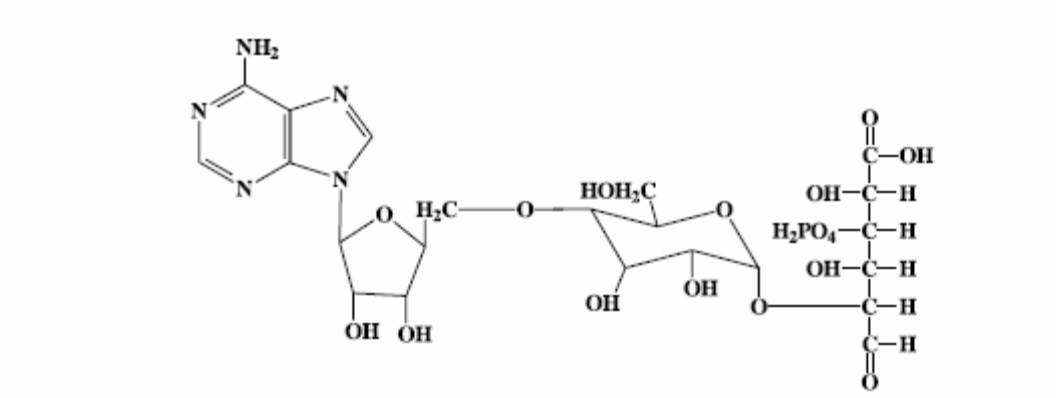
Figure 1. Mechanism of toxicity of Bt.

The specific receptors in the insect midgut, necessary for binding of the δ-endotoxin do not exist in non-target vertebrates like fish, birds, mammals and humans.
A large number of related crystal proteins are known and more than one protein type may be present in one crystal. The exact type of protein determines which insect pest species will be killed.
The description of Bt products for registration is based on the types of **δ-endotoxin** proteins.

2. ß-exotoxin (beta-exotoxin)

This toxin, also called thuringiensin, has been detected in strains from several subspecies, e.g.
B. thuringiensis subspecies darmstadiensis,
B. thuringiensis subspecies galleriae,
B. thuringiensis subspecies thuringiensis,
and *B. thuringiensis* subspecies tenebrionis.

The toxin is formed during vegetative growth of Bt.
It is thermo stable (resists 70°C for 15 minutes), and has a broad spectrum of activity. It inhibits the biosynthesis of RNA by acting competitively with ATP. RNA synthesis is a vital process in all life, so **beta-exotoxin is toxic towards almost all forms of life.**



The structure of thuringiensin. It is an adenine nucleotide derivative containing adenosine, glucose and allaric acid.

3. Other toxins and exo-enzymes that might occur

- α-Exotoxin (alpha-exotoxin): this heat-lable toxin is known to be toxic to some non-target insects, fish and mammals.
- Enterotoxin: An indication exists that registered *Bacillus thuringiensis* products may be able to produce the diarrheal enterotoxin usually associated with *Bacillus cereus.*
- Exo-enzymes: Several Bt and Bc enzymes have been described which may play a role in non-target activity: phospholipase, sphingomyelinase, protease, chitinase, and haemolysin.

Like ß-exotoxin, these toxins are produced during vegetative growth

The manifestation of one or more of these toxins appears to be at least partly related to production methodology, especially the composition of the growth media used in industrial fermentation.

Glossary.

Exotoxin	A toxin that is excreted by a microorganism
Endotoxin	A toxin that remains in the cell of the microorganism until the cell is lysed or during bacterial cell division
Enterotoxin	A toxin that causes vomiting, diarrhea and abdominal pain to humans, like food-poisoning

Production of *Bacillus thuringiensis* based insecticides

Examples of some Bt insecticides, registered in the Europe or the USA are Delfin, DiPel, DiPel ES, Scutello, Scutello L, Turex 50 WP and XenTari WG.
Each product is characterized by its **delta-endotoxins,** produced by the specific isolate of *Bacillus thuringiensis* used.
Each product has its specific range of target pest insects.

Formulations include Water Dispersible Granule, Dry Flowable, Aqueous Suspension, Granule, Technical Powder, Dust, Wettable Powder, Emulsifiable Suspension, Aqueous Flowable, Bait, and Oil Flowable.

Description of the product BN3, Bitoxibacillin P

Bitoxibacillin is a Russian bacterial insecticide, based on *Bacillus thuringiensis* var thuringiensis. The active ingredients are not only the spores and δ-endotoxins, but also **ß-exotoxin**.

Health risks and registration of *Bacillus thuringiensis* based insecticides

Bt insecticides are used all over the world, and are considered to be safe for non-target insects, for other non-vertebrate and vertebrate animals and humans. However a restriction has to be made for products that contain **ß-exotoxin**.

Studies of the acute toxicity of ß-exotoxin are few and inconclusive. Acute oral LD50 of thuringiensin in rats has been reported to range from 170 mg/kg to over 1090 mg/kg or even over 5000 mg/kg. The acute inhalation LC50 in rats ranges from 0.024 mg/l to over 0.3 mg/l for 4 h of exposure.

Out of concern for mammalian toxicity, the World Health Organization (1999) addressed non-specific thuringiensin from different subspecies of Bt to promote human health, and regulated Bt strains that produced thuringiensin. Furthermore, for convenience and economy, most Bt or thuringiensin products are wettable powders can easily enter the airways of vertebrates, putting at risk the health of production workers and field workers.

> vegetative growth, various Bt strains are capable of producing an assortment of antibiotics, enzymes, secondary metabolites and toxins, including Bc toxins, that may have detrimental effects on both target organisms and non-target organisms. Of particular note **is beta-exotoxin**.

In the United States the Environmental Protection Agency requires the following risk mitigation measures:

1. Production batch testing, in order to detect undesirable toxins and to detect contamination by pathogenic bacteria.
2. If the organism is capable of producing beta-exotoxin, the registrant must ensure that none is present in the Technical Grade Active Ingredient and that the product is not put in a medium, including formulated end use products that allows germination and/or growth at any time prior to use.

So far etectable levels of ß-exotoxin are not allowed in B. thuringiensis products in Western Europe and North America.

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