Bacillus thuringiensis

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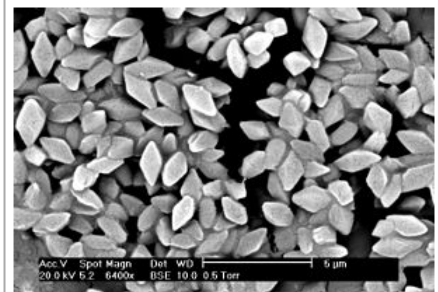
Bacillus thuringiensis (or **Bt**) is a Gram-positive, soil-dwelling bacterium, commonly used as a biological pesticide; alternatively, the Cry toxin may be extracted and used as a pesticide. *B. thuringiensis* also occurs naturally in the gut of caterpillars of various types of moths and butterflies, as well on leaf surfaces, aquatic environments, animal feces, insect rich environments, flour mills and grain storage facilities.[1][2]

During sporulation, many Bt strains produce crystal proteins (proteinaceous inclusions), called δ-endotoxins, that have Insecticide action. This has led to their use as insecticides, and more recently to genetically modified crops using Bt genes. Many crystal-producing Bt strains, though, do not have insecticidal properties.[3]

Bacillus thuringiensis



Spores and bipyramidal crystals of *Bacillus thuringiensis* morrisoni strain T08025

Scientific classification

Domain:	Bacteria
Phylum:	Firmicutes
Class:	Bacilli
Order:	Bacillales
Family:	Bacillaceae
Genus:	*Bacillus*
Species:	***B. thuringiensis***

Binomial name

Bacillus thuringiensis

Berliner 1915

Contents

Discovery and mechanism of insecticidal action

B. thuringiensis was first discovered in 1901 by Japanese biologist Ishiwata Shigetane.[3] In 1911, *B. thuringiensis* was rediscovered in Germany by Ernst Berliner, who isolated it as the cause of a disease called *Schlaffsucht* in flour moth caterpillars. In 1976, Robert A. Zakharyan reported the presence of a plasmid in a strain of *B. thuringiensis* and suggested the plasmid's involvement in endospore and crystal formation.[4][5] *B. thuringiensis* is closely related to *B.cereus*, a soil bacterium, and *B.anthracis*, the cause of anthrax: the three organisms differ mainly in their plasmids.[6]:34-35 Like other members of the genus, all three are aerobes capable of producing endospores.[1] Upon sporulation, *B. thuringiensis* forms crystals of proteinaceous insecticidal δ-endotoxins (called crystal proteins or Cry proteins), which are encoded by *cry* genes.[7] In most strains of *B. thuringiensis*, the *cry* genes are located on a plasmid (in other words, *cry* is not a chromosomal gene in most strains).[8][9][10]

Cry toxins have specific activities against insect species of the orders Lepidoptera (moths and butterflies), Diptera (flies and mosquitoes), Coleoptera (beetles), Hymenoptera (wasps, bees, ants and sawflies) and nematodes. Thus, *B. thuringiensis* serves as an important reservoir of Cry toxins for production of biological insecticides and insect-resistant genetically modified crops. When insects ingest toxin crystals, the alkaline pH of their digestive tract denatures the insoluble crystals, making them soluble and thus amenable to being cut with proteases found in the insect gut, which liberate the cry toxin from the crystal.[8] The Cry toxin is then inserted into the insect gut cell membrane, paralyzing the digestive tract and forming a pore.[11] The insect stops eating and starves to death; live Bt bacteria may also colonize the insect which can contribute to death.[11][8][12] Research published in 2006 has suggested the midgut bacteria of susceptible larvae are required for *B. thuringiensis* insecticidal activity.[13]

In 1996 another class of insecticidal proteins in Bt was discovered; the vegetative insecticidal proteins (Vip).[14][15] Vip proteins do not share sequence homology with cry proteins, in general do not compete for the same receptors, and some kill different insects than do cry proteins.[14]

In 2000, a novel functional group of Cry protein, designated parasporin, was discovered from non-insecticidal *B. thuringiensis* isolates.[16] The proteins of parasporin group are defined as *Bacillus thuringiensis* and related bacterial parasporal proteins that are non-hemolytic but capable of preferentially killing cancer cells.[17] As of January 2013, parasporins comprise six subfamilies (PS1 to PS6).[18]

Use of spores and proteins in pest control

Spores and crystalline insecticidal proteins produced by *B. thuringiensis* have been used to control insect pests since the 1920s and are often applied as liquid sprays.[19] They are now used as specific insecticides under trade names such as DiPel and Thuricide. Because of their specificity, these pesticides are regarded as environmentally friendly, with little or no effect on humans, wildlife, pollinators, and most other beneficial insects and are used in Organic farming,[20] however the manuals for these products do contain many environmental and human health warnings,[21][22] and a 2012 European regulatory peer review of 5 approved strains found that while there is data to support some claims of low toxicity to humans and the environment, there is insufficient data to justify many of these claims.[23]

Bacillus thuringiensis serovar *israelensis*, a strain of *B. thuringiensis* is widely used as a larvicide against mosquito larvae, where it is also considered an environmentally friendly method of mosquito control.[24]

As, for example, insects develop resistance to Bt,[25] or there is desire to force mutations to modify organism characteristics[26] or to use homologous recombinant genetic engineering to improve crystal size and increase pesticidal activity[27] or broaden the host range of Bt and obtain more effective formulations,[28] etc., new strains of Bt are developed and introduced over time.[29] Each new strain is given a unique number and registered with the U.S. EPA[30] and allowances may be given for genetic modification depending on "its parental strains, the proposed pesticide use pattern, and the manner and extent to which the organism has been genetically modified".[31] Formulations of Bt that are approved for organic farming in the US are listed at the website of the Organic Materials Review Institute (OMRI)[32] and several university extension websites offer advice on how to use Bt spore or protein preparations in organic farming.[33][34]

Use of Bt genes in genetic engineering of plants for pest control

The Belgian company Plant Genetic Systems (now part of Bayer CropScience) was the first company (in 1985) to develop genetically engineered (tobacco) plants with insect tolerance by expressing *cry* genes from *B. thuringiensis*.[35][36] The Bt tobacco was never commercialized; tobacco plants are used to test genetic modifications since they are easy to manipulate genetically and are not part of the supply.[37][38]

Usage

In 1995, potato plants producing CRY 3A Bt toxin were approved safe by the Environmental Protection Agency, making it the first pesticide-producing crop to be approved in the USA.[40][41] This was the "New Leaf" potato, and it was removed from the market in 2001 due to lack of interest.[42] For current crops and their acreage under cultivation, see genetically modified crops.

In 1996, genetically modified maize producing Bt cry protein was approved, which killed the European corn borer and related species; subsequent Bt genes were introduced that killed corn rootworm larvae.[43]

The Bt genes that have been engineered into crops and approved for release include the following, singly and stacked: Cry1A.105, CryIAb, CryIF, Cry2Ab, Cry3Bb1, Cry34Ab1, Cry35Ab1, mCry3A, and VIP, and the engineered crops include corn and cotton.[44][45]:285ff Corn genetically modified to produce VIP was first approved in the US in 2010.[46] Monsanto developed a soybean expressing Cry1Ac and the glyphosate-resistance gene for the Brazilian market, which completed the Brazilian regulatory process in 2010.[47][48]

Insect resistance

In November 2009, Monsanto scientists found the pink bollworm had become resistant to the first generation Bt cotton in parts of Gujarat, India - that generation expresses one Bt gene, *Cry1Ac*. This was the first instance of Bt resistance confirmed by Monsanto anywhere in the world.[49][50] Monsanto immediately responded by introducing a second generation cotton with multiple Bt proteins, which was rapidly adopted.[49] Bollworm resistance to first generation Bt cotton was also identified in Australia, China, Spain and the United States.[51]

Bt toxins present in peanut leaves (bottom image) protect it from extensive damage caused by Lesser Cornstalk Borer larvae (top image).[39]

Secondary pests

Several studies have documented surges in "sucking pests" (which are not affected by Bt toxins) within a few years of adoption of Bt cotton. In China, the main problem has been with mirids,[52][53] which have in some cases "completely eroded all benefits from Bt cotton cultivation”.[54] A 2009 study in China concluded that the increase in sucking pests depended on local temperature and rainfall conditions and increased in half the villages studied. The increase in insecticide use for the control of these secondary insects was far smaller than the reduction in total insecticide use due to Bt cotton adoption.[55] Another study published in 2011 was based on a survey of 1,000 randomly selected farm households in five provinces in China and found that the reduction in pesticide use in Bt cotton cultivars is significantly lower than that reported in research elsewhere, consistent with the hypothesis suggested by recent studies that more pesticide sprayings are needed over time to control emerging secondary pests, such as aphids, spider mites, and lygus bugs.[56]

Similar problems have been reported in India, with both mealy bugs[57][58] and aphids[59] although a survey of small Indian farms between 2002 and 2008 concluded that Bt cotton adoption has led to higher yields and lower pesticide use, decreasing over time.[60]

Controversies

Main article: Genetically modified food controversies

Kenyans examining insect-resistant transgenic Bt corn

There are controversies around GMOs on several levels, including whether making them is ethical, whether food produced with them is safe, whether such food should be labeled and if so how, whether agricultural biotech is needed to address world hunger now or in the future, and more specifically to GM crops—intellectual property and market dynamics; environmental effects of GM crops; and GM crops' role in industrial agricultural more generally.[61]

There are also issues specific to Bt transgenic crops.

Lepidopteran toxicity

The most publicised problem associated with Bt crops is the claim that pollen from Bt maize could kill the monarch butterfly.[62] The paper produced a public uproar and demonstrations against Bt maize; however by 2001 several follow-up studies coordinated by the USDA had proven that "the most common types of Bt maize pollen are not toxic to monarch larvae in concentrations the insects would encounter in the fields."[63][64][65][66]

Wild maize genetic mixing

A study published in *Nature* in 2001 reported that Bt-containing maize genes were found in maize in its center of origin, Oaxaca, Mexico.[67] In 2002 *Nature* "concluded that the evidence available is not sufficient to justify the publication of the original paper."[68] A significant controversy happened over the paper and *Nature*'s unprecedented notice.[69][70]

A subsequent large-scale study, in 2005, failed to find any evidence of genetic mixing in Oaxaca.[71] A 2007 study found that "transgenic proteins expressed in maize were found in two (0.96%) of 208 samples from farmers' fields, located in two (8%) of 25 sampled communities. Mexico imports a substantial amount of maize from the US, and due to formal and informal seed networks among rural farmers, there are many potential routes of entrance for transgenic maize into food and feed webs."[72] A study published in 2008 showed some small-scale (about 1%) introduction of transgenic sequences in sampled fields in Mexico; it did not find evidence for or against this introduced genetic material being inherited by the next generation of plants.[73][74] That study was immediately criticized, with the reviewer writing that "Genetically any given plant should be either non-transgenic or transgenic, therefore for leaf tissue of a single transgenic plant, a GMO level close to 100% is expected. In their study, the authors chose to classify leaf samples as transgenic despite GMO levels of ~0.1%. We contend that results such as these are incorrectly interpreted as positive and are more likely to be indicative of contamination in the laboratory."[75]

Disproven link to colony collapse disorder

As of 2007, a new phenomenon called colony collapse disorder (CCD) began affecting bee hives all over North America. Initial speculation on possible causes ranged from new parasites to pesticide use[76] to the use of Bt transgenic crops.[77] The Mid-Atlantic Apiculture Research and Extension Consortium published a report in March 2007 that found no evidence that pollen from Bt crops is adversely affecting bees.[78] The actual cause of CCD was unknown in 2007, and scientists believe that it may have multiple exacerbating causes.[79] A leading theory as of January 2013 was that neonicotinoids may be the cause.[80][81]

Beta-exotoxins

Some isolates of *B. thuringiensis* produce a class of insecticidal small molecules called beta-exotoxin, the common name for which is thuringiensin.[82] A consensus document produced by the OECD says: "Beta-exotoxin and the other Bacillus toxins may contribute to the insecticidal toxicity of the bacterium to lepidopteran, dipteran, and coleopteran insects. Beta-exotoxin is known to be toxic to humans and almost all other forms of life and its presence is prohibited in B. thuringiensis microbial products. Engineering of plants to contain and express only the genes for δ-endotoxins avoids the problem of assessing the risks posed by these other toxins that may be produced in microbial preparations."[83]

See also

- Biological insecticides
- Genetically modified food
- Western corn rootworm

An **Ovitrap**, a tool for the collection of eggs from tiger mosquitoes. In this case, an ovitrap type used for the monitoring of the Asian Tiger mosquito *Aedes albopictus* in the Swiss canton of Ticino. The presence of the mosquitoes is detected through the eggs they lay on the wooden paddle or from larvae that hatch from these eggs in the laboratory. The brown granules in the water are a *Bacillus thuringiensis israelensis preparation that will kill mosquito larvae that hatch in the ovitrap. Ovitraps are also used to monitor the yellow fever mosquito* Aedes aegypti.

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External links

- Bacillus thuringiensis General Fact Sheet (http://npic.orst.edu/factsheets/BTgen.pdf) (National Pesticide Information Center)
- Bacillus thuringiensis Technical Fact Sheet (http://npic.orst.edu/factsheets/BTtech.pdf) (National Pesticide Information Center)
- Breakdown of the Bt toxin and effects on the soil quality (http://www.gmo-safety.eu/database/1004.breakdown-toxin-effects-micro-organisms-soil.html) Research project and results
- The Bacillus thuringiensis Toxin Specificity Database (http://cfs.nrcan.gc.ca/subsite/glfc-bacillus-thuringiensis/bacillus-thuringiensis) at Natural Resources Canada
- *Bacillus thuringiensis* Taxonomy (NIH) (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=1428)
- Bacillus thuringiensis (http://patricbrc.org/portal/portal/patric/Taxon?cType=taxon&cId=1428) genomes and related information at PATRIC (http://patricbrc.org/), a Bioinformatics Resource Center funded by NIAID (http://www.niaid.nih.gov/)
- bEcon - Economics literature about the impacts of genetically engineered (GE) crops in developing economies (http://www.mendeley.com/groups/1296883/becon)