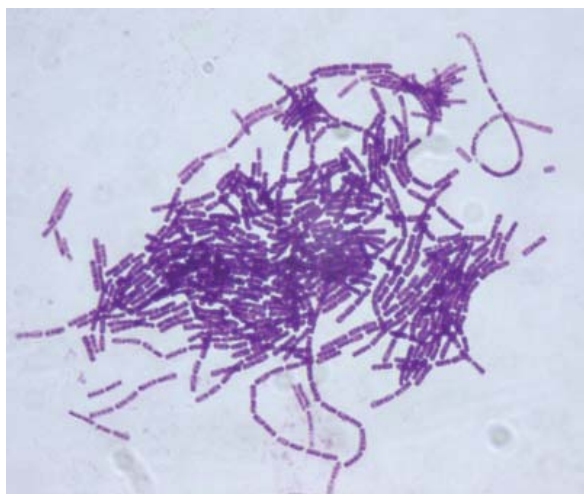


***Bacillus thuringiensis* (Bt)**



Gram stain of *Bacillus thuringiensis* under 1000 X magnification by Dr. Sahay on commons.wikimedia.org

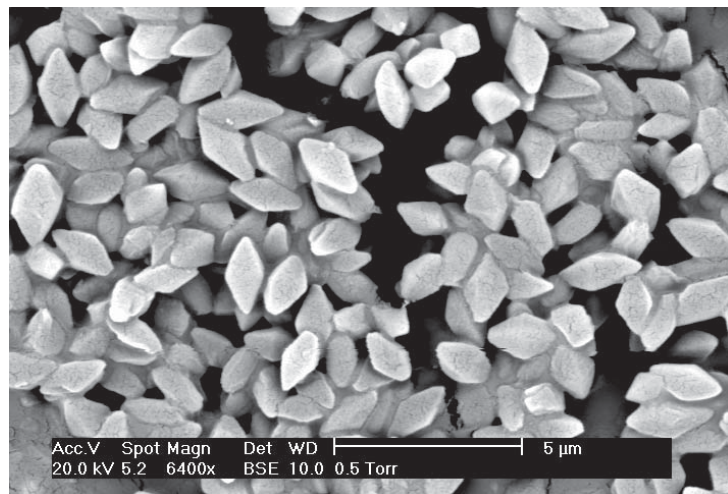
where a diseased Mediterranean flour moth – from which Ernst Berliner isolated the bacterium – was found in 1911.

Bacillus thuringiensis – frequently referred to by its initials of Bt – is a naturally occurring, ubiquitous species of spore-forming, rod-shaped bacterium that is used as a biological pesticide for insect control. Bacteria in the genus *Bacillus* commonly occur in soils, and most types with insecticidal activity have been isolated from soil samples. Bt can be found almost everywhere in the world, sparsely distributed in the soil in all types of habitats, including beaches, desert, and tundra.

This species of bacterium was first recognized in 1901 when a Japanese biologist, Shigetane Ishiwatari, isolated it as the cause of the disease that was killing large populations of silkworms. The name he gave this species, *Bacillus sotto*, was later ruled invalid and we now know this bacterium as *Bacillus thuringiensis*, after the German town of Thuringia

Farmers in Europe had started to use Bt as a pesticide by 1920, with a commercial spore-based formulation in France called Sporine in 1938 used primarily to kill flour moths.

When producing its spores, many Bt strains make crystal proteins, which Berliner had noted in 1915. It wasn't until 1956 that researchers discovered that the main insecticidal activity was due to those proteins. These proteins are toxins – called endotoxins – that kill some insects, but not others. Different endotoxins may be active against an entire order of insects, or they may be effective against only one or a few species. These proteins are not toxic to humans or other mammals, and little to no direct toxicity to non-target insects or other wildlife has been observed. It is these proteins that are the basis of the insecticidal activity of the bacterium.



Crystals of Bt-toxin from *Bacillus thuringiensis*. Microscopy by Jim Buckman on commons.wikimedia.org

In the US Bt is the most widely used microbial insecticide. It was used commercially starting in 1958 and has been registered for use in pesticides by the US Environmental Protection Agency (EPA) since 1961, and now Bt strains are found in over 180 registered pesticide products. Some are for use on crops and ornamental plants, while others are used in and around buildings, in aquatic settings, and in aerial applications. Some of these products are approved for use in organic production.

Bacterial insecticides must be eaten by target insects to be effective; they are not contact poisons. When the bacterium is ingested by a susceptible insect, the high pH and enzymatic activity in the insect's gut dissolves the crystal, releasing the proteins which bind to specific receptors in the gut.



Soybean loopers infected with Bt. Bottom: healthy larva. Middle: larva infected for 24 hours appears healthy, but is very sluggish and has stopped feeding. Top: dead larva 48 hours after initial infection. Photo by Daniel L. Mahr.

The toxins paralyze and destroy the cells of the gut wall, allowing the gut contents to enter the insect's body cavity. Poisoned insects stop feeding within hours, may die quickly from the activity of the toxin or may die within 2 or 3 days from the effects of septicemia (blood-poisoning). These toxins are not activated in mammals, so they cannot harm humans. (Related bacteria, such as *B. cereus*, produce toxins that do cause gastroenteritis (food poisoning) in humans.)

Bt does not reproduce and persist in the environment in sufficient quantities to provide continuing control of target pests. Spores do not spread to other insects or cause disease outbreaks on their own. The bacteria may multiply in the infected host, but because few spores or crystal protein toxins are produced, few infective units are released when a poisoned insect dies. Bt cells last only a few days on the soil surface (although they can survive for several months when covered by soil) and the toxins are rapidly broken down by sunlight and other

microbes. Consequently, Bt products are applied much like synthetic insecticides. Bt treatments are inactivated within one to a few days in many outdoor situations, and repeated applications may be necessary for some crops and pests.

Bt products are produced commercially in large industrial fermentation tanks. The bacterial cells usually produce a spore and endotoxin as they develop. Most commercial Bt products contain the protein toxin and spores, but some contain only the toxin component.

Each type of Bt creates endotoxins that bind to different receptors in the gut, so can only be activated by certain insect larvae, so each isolate or subspecies is highly specific to different target insects. There are thousands of different Bt strains, producing over 200 proteins that affect a wide range of insects and some other invertebrates. Initially the strains of Bt known were effective only against caterpillars, but isolates that kill other types of pests were eventually identified and developed, with the first subspecies toxic to flies discovered in 1977 and strains toxic to beetles in 1983. Bt formulations that are commercially available fall into the following broad categories.

- **Bt Formulations That Kill Caterpillars.** The best-known and most widely used Bt insecticides are formulated from *Bacillus thuringiensis* var. *kurstaki* (Btk) isolates that are pathogenic and toxic only to larvae of the butterflies and moths (Lepidoptera). Many such Bt products have been registered under a variety of trade names. They are used to control many common leaf-feeding caterpillars, including pests on vegetables, especially the “worms” that attack cabbage, broccoli, cauliflower, and Brussels sprouts; bag worms and tent caterpillars on trees and shrubs; larvae of the gypsy moth and other forest caterpillars; and European corn borer larvae in field corn. Some products are used to control Indian meal moth larvae in stored grain.

Bacillus thuringiensis var. *aizawai* is another Bt that kills caterpillars. It produces slightly different toxins and is also highly toxic to honeybees.



Thuricide is one of several brands of Bt used for caterpillar control.

Bt products that kill caterpillars are not effective against other types of pests. Even certain caterpillars are not controlled by Bt, especially those that live in the soil or bore into plant tissues without consuming a significant amount of the Bt applied to plant surfaces. The peach tree borer in stone fruits, corn earworm in corn, and cutworms that clip off garden plants are examples of caterpillars

seldom controlled by Bt treatments. Most Bt products are not labeled for the control of codling moth larvae that attack apples and pears because these larvae do not feed on fruit surfaces.

■ **Bt Formulations That Kill Fly Larvae.** *Bacillus thuringiensis* var. *israelensis* (Bti) kills the larvae of certain flies and mosquitoes – before they become biting adults. The main targets for this Bt are the larval stages of mosquitoes, black flies, and fungus gnats; it does not kill larval stages of other flies such as the house fly, stable fly, or blow flies. *Aedes* and *Psorophora* are the most susceptible mosquito genera; *Anopheles* and *Culex* species require higher than normal rates of Bti. Bti is most effective for mosquito or black fly control when it is used on a community-wide basis by mosquito abatement district personnel. For most homeowners, eliminating sites that periodically serve as sources of standing water (such as tires, birdbaths and empty containers) and controlling weeds around stagnant ponds or drainage lagoons is more effective than applying Bti. It is very useful in rain barrels, water gardens, and other places where standing water is wanted. Bti is not very effective for the control of mosquito larvae in turbid water or waters containing high levels of organic pollutants.



An example of a granular formulation of Bti.

Some Bti products are used effectively for the control of fungus gnat larvae in greenhouses and in mushroom production. For these uses, Bti is applied as a drench to potting soils or culture media.

■ **Bt Formulations That Kill Beetles.** Another group of Bt isolates, including those from *Bacillus thuringiensis* var. *san diego* and *Bacillus thuringiensis* var. *tenebrionis*, are toxic to certain beetles. Within the order Coleoptera (the beetles), species exhibit great differences in susceptibility to these isolates, presumably because of differences in the receptor sites in the gut wall of the insects where the bacterial toxins must attach. Consequently, the range of susceptible hosts for the beetle-targeted Bt formulations does not include all beetles, or even all of the species within a family or subfamily. Some of the common pests these Bt formulations are effective against include larvae of Colorado potato beetle and adults and larvae of elm leaf beetle and willow leaf beetle. Homeowner products may not be available.

In addition to Bt products used by homeowners, advances in molecular biology and biotechnology led to Bt crop plants, where genes directing the production of Bt toxins are incorporated into plants so that the Bt toxin is produced within the plant. Tobacco was the first modified plant since it is easy to manipulate genetically, but that was never developed as a commercial product. New Leaf potato was the first commercial genetically engineered plant, registered with the EPA in 1995 (but taken off the market in 2001 due to lack of sales), followed by Bt-corn in 1996, and later Bt cotton and soybeans. Dietary risk and allergenicity studies showed no observed adverse effects, and ecological risk assessments have shown that non-target caterpillars, such as monarch butterflies, are not exposed to enough toxin to affect them. However, the season-long, high-level control Bt crops can provide poses a great risk for the development of insect resistance to the Bt toxin, and some insects have developed resistance to Bt crops. The use of Bt crops initially results in dramatically reduced insecticide use, but in many cases more pesticide sprayings are needed over time to control emerging secondary pests. The use of these transgenic crops is still controversial because of these factors, as well as the potential for unintended environmental consequences not recognized yet.

Using Bt Insecticides. Insecticides containing Bt can be very effective for insect control in a variety of situations. Since each Bt insecticide controls only certain types of insects, it is essential to correctly identify the target pest and to confirm that the Bt product label states that the insecticide is effective

against that particular pest. Separate stages of insects differ in their susceptibility to Bt; isolates that are effective against larval stages of butterflies, moths, or mosquitoes generally do not kill adults. Bt is most effective when applied to caterpillars during their 1st and 2nd instars, when they are still small. Because susceptible insects must consume Bt to be poisoned, treatments must be directed to the plant parts that the target pest will eat. Poisoned insects normally remain on plants for a day or two after treatment, but they do not continue feeding and will soon die. Where Bt applied to plant surfaces or other sites is exposed to sunlight, it is deactivated rapidly by direct ultraviolet radiation. To maximize the effectiveness of Bt treatments, sprays should thoroughly cover all plant surfaces, including the undersides of leaves. Treating in the late afternoon or evening can be helpful because the insecticide remains effective on foliage overnight before being inactivated by exposure to intense sunlight the following day. Treating on cloudy (but not rainy) days provides a similar result. Production processes that encapsulate Bt spores or toxins in a granular matrix (such as starch) or within killed cells of other bacteria also provide protection from ultraviolet radiation.

Bt products are available as sprays, dusts, granules, and pellets. Dunks (right) are small donuts or briquettes containing slow-release formulas of Bti that float on the water surface, slowly dissolving to release the insecticide for up to 30 days. Users are advised to handle all microbial insecticides cautiously even though this bacterium is non-toxic to humans. Bacterial spores, mold spores, and virus particles become “foreign proteins” if they are inhaled or rubbed into the skin and can cause allergic reactions. The dusts or liquids used to dilute and carry these microorganisms also can act as allergens or irritants. These problems do not prevent the safe use of microbial insecticides, but users should not breathe dusts or mists of microbial insecticides. Users should wear gloves, long sleeves, and long trousers during application and wash thoroughly afterwards. These are common-sense precautions that will help prevent unexpected reactions and minimize any effects from unknown toxicity.



Mosquito Dunks are one brand of slow-release Bti.

In addition to *B. thuringiensis*, there are about 100 species of *Bacillus* reported to kill insects. Several have been used for insect control, including:

- *Bacillus popilliae* var. *popilliae* and *B. lentimorbus* cause milky disease which kills Japanese beetle larvae (*Popillia japonica*) that infest lawns in much of the Midwest. It is not effective against the closely related annual white grubs (masked chafers). The bacteria spread naturally when infected beetle larvae die, releasing billions of new spores into the soil as the insect decomposes, so only a single application is usually necessary (although it may take a long time for the population to build up to sufficient levels in the soil. This was the first microbial insecticide to be developed commercially in the US.
- *Bacillus sphaericus* is especially active against larvae of mosquitoes in the genera *Culex*, *Psorophora*, and *Culiseta*, remaining effective in stagnant or turbid water. One strain of *B. sphaericus* was registered by the U.S. EPA in 1991.



One brand of milky spore powder.

– Susan Mahr, University of Wisconsin – Madison

Additional Information:

- *Bacillus thuringiensis* (Bt) – on the national Pesticide Information Center (NPIC) website at npic.orst.edu/ingred/bt.html
- *Bacillus thuringiensis* – a website by University of California San Diego at www.bt.ucsd.edu/index.html