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Bioprospecting of *Bacillus thuringiensis* in the control of *Aedes aegypti* larvae

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Abstract. *Aedes aegypti* (Diptera: Culicidae) is the main vector of Dengue, Zika and Chikungunya, diseases that have attracted the attention of scientific society and the population in general, due to epidemiological outbreaks and numerous health hazards and the imminent risk of death. Much has been discussed about more efficient forms of control for this mosquito, considering that the chemical control, currently used, has been causing impacts on the environment and the health of the population. Thus, alternative methods have been evaluated. Among them, biological control through products formulated from *Bacillus thuringiensis* has stood out, as biotechnological advances have allowed to improve and enhance the products already on the market, as well as to develop new bioinsecticides from the entomopathogenic proteins produced by these microorganisms, aiming to make the larvae control more and more effective. To this end, this study aimed to carry out a bibliographic survey on the use of *Bacillus thuringiensis* as a form of biological control of *Aedes aegypti* larvae, due to the need to use safer and more effective methods of control for disease vector insects.

Keywords: Biological control; Dengue; Chikungunya; Zika; Cristals; Culicid.

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Introduction

The genus *Aedes* has several species distributed throughout the world. Among them, *Aedes aegypti* (Diptera: Culicidae) stands out, mosquitoes abundant throughout Brazil and distributed in approximately 4,523 municipalities (Zara et al., 2016). They are important vectors of arboviruses that cause diseases such as dengue, chikungunya, zika and yellow fever (Miller and Ballinger, 1988; Lourenço-de-Oliveira et al., 2004).

The ease with which populations of this culicid increase and remain in urban centers is worrying, because, when they reproduce, they use several places that contain standing water to lay eggs, which, because they are resistant to desiccation, can remain viable in dry places for more than a year (Consoli and Lourenço-de-Oliveira, 1994).

Due to the rapid proliferation of mosquitoes, the number of people infected by the arboviruses transmitted by them has increased significantly. This has a great impact on public health, requiring the development of preventive measures, as well as the effective control of these vectors.

The use of chemical insecticides is the most used method to control these mosquitoes, however, problems related to the environment and the health of the population have led researchers to seek alternative ways to eradicate them. In this sense, biological control through the use of entomopathogenic bacteria or formulated from them, can be an important ally in the fight against *A. aegypti* larvae.

The *Bacillus* genus is a large group that comprises several species of bacteria, such as *B. alvei*, *B. circulans*, *B. macerans*, *B. cereus*, *B. amyloliquefaciens*, *B. subtilis*, *B. pumilis*, *B. licheniformis*, *B. megaterium*, *B. flexus*, *B. fimus*, *B. neurolyticus*, *B. brevis*, *B. sphaericus*, *B. lentus*, *B. coagulans*, *B. stearothermophilus* and *B. thuringiensis* (Barros, 2004). Among them, *B. thuringiensis* (Bt) has a high degree of toxicity for larvae of different insect species, and can also be used together with chemicals to increase control efficiency (Chui et al., 1995; Polanczyk et al., 2003), without causing a reduction in the biodiversity of the treated sites (Becker, 2000).

Therefore, this work aimed to carry out a bibliographic survey on the efficiency of *B. thuringiensis* (Bt) in the control of *A. aegypti* larvae, a vector of several arboviruses that have an impact on health and society. Scientific data is necessary for the development of new technologies to be used to benefit the environment and the world population.

Aedes aegypti

Aedes aegypti Linnaeus, 1762 (Diptera: Culicidae) is originally from Africa. However, as a result of the destruction of natural habitats, they would have gone through a process of genetic selection, and selected varieties found, in altered areas and in human clusters, environments suitable for their survival (Viveiros, 2010). From Africa, they migrated to Southern Europe and Asia by sea transport (Lounibos, 2002) and spread throughout the world.

Part of the life cycle occurs in water and, therefore, they have great adaptive capacity to the type and quality of the reservoirs where they develop. Thus, although they can develop in polluted waters, containing different concentrations of organic matter, including domestic sewage (Beserra et al., 2009), they prefer cleaner waters found in household and household containers including unused tires, cans, bottles, dishes with vase, open water tanks, unused pools, among others (Neves, 2005).

Currently, it is considered cosmopolitan. It has a wide distribution, being found both in urban and suburban areas of tropical and subtropical regions, between latitudes 35° N and 35° S, as well as, latitude up to 45° N (Viveiros, 2010). They present complete metamorphosis and the life cycle comprises four phases: egg, larva (4 instars), pupa and the adult (Figure 1) (Gadelha and Toda, 1985; Araújo, 2011).

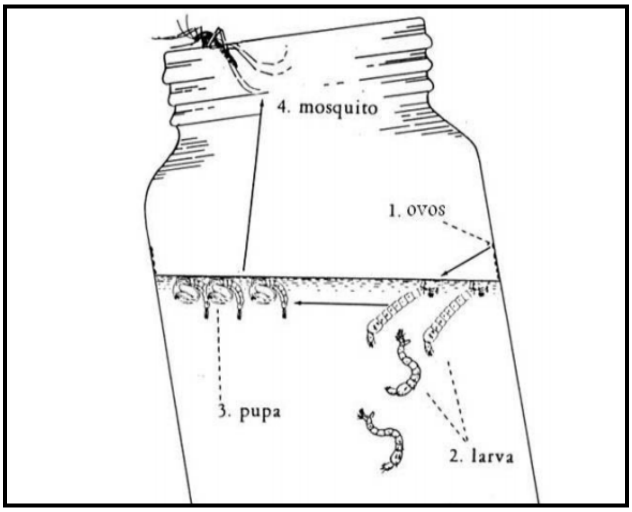

Figure 1. Life cycle of culicids. 1 - egg, 2 - larva (4 instars), 3 - pupa, 4 - adult mosquito. Source: Adapted from Araújo (2011).

Upon contact with water, the larvae hatch from the eggs, passing through four instars (L1, L2, L3 and L4). The duration of each instar varies according to the temperature, availability of food and larval density at the breeding site (Araújo, 2011).

These larvae feed mainly on aquatic microorganisms (phytoplankton, zooplankton and benthos), as well as sedimented matter. At the end of the 4th instar, they accumulate nutritional reserves and become pupae. At this stage, insects do not feed and undergo the necessary changes that will make them reach adulthood, in which the transition from aquatic to terrestrial habitat occurs (Marcondes, 2011).

Adults are invertebrate metazoans, with bilateral symmetry, chitinous exoskeleton, segmented body and appendages articulated in pairs (Viveiros, 2010). According to Araújo (2011), it presents a dark coloring, white spots on the body and a lyre-shaped design present on its back, a characteristic that facilitates its identification with the naked eye.

The female of *A. aegypti* feeds on blood so that the maturation of the eggs can occur, and the hematophagy usually occurs during the day. Due to the need for blood meal, the female can disperse up to 800 m in search of food, being able to ingest from 3.0 to 3.5 mg of blood in each food (Araújo, 2011).

In each oviposition, you can place around 50 to 200 eggs in water tanks. However, it is capable of performing numerous postures throughout its life, as it copulates only once with each male, and stores sperm in its spermatheque, where the sperm remains from insemination until before the fertilization of the eggs. When the eggs complete their ripening (gonadotrophic cycle), the female is able to lay. (Gadelha and Toda, 1985; Araújo, 2011).

The eggs of these culicids remain in a state of dormancy during the egg phase (quiescence), which allows it to have a pause in embryonic development, especially when environmental conditions are unfavorable (Azevedo, 2015).

Due to this resistance to variations in the environment and relatively short life cycle, the mosquito has spread rapidly. Infestations have become one of the main public health problems (Braga and Valle, 2007; Marchioro, 2016), given that this species is responsible for the transmission of diseases such as Dengue, Yellow Urban Fever (FAU), Chikungunya Fever and a Zika virus fever (Black et al., 2002; Luz et al., 2015).

Dengue, known worldwide for affecting a large number of people, is transmitted by this culicid. The records of the first infestations of the dengue virus are dated to the end of the 13th century, in Java, an island in Southeast Asia and in Philadelphia, in the United States. However, only from the 20th century, the World Health Organization (WHO) started to recognize it as a disease (Ragonha and Nowak, 2018).

Dengue epidemics have occurred in Brazil since 1986 and have been caused by four serotypes of the DENV virus (DENV-1, 2, 3 and 4), belonging to the genus *Falvivirus* and family Flaviviridae (Braga and Valle, 2007; Marchioro, 2016). This disease has as main symptoms, an abrupt high fever, headache, pain behind the eyes, muscle and joint pain, skin rashes, which can cause death in cases of hemorrhagic dengue (Polanczyk et al., 2003).

Yellow fever is caused by an arbovirus of the genus Flavivirus, family Flaviviridae. The first outbreak recorded in Brazil occurred in the mid-eighteenth century, in the northeast region (Costa et al., 2018). According to the Brazilian Society of Infectious Diseases (SBI) the symptoms are classified as mild, moderate or severe, as follows:

> **a) Light form:** The clinical picture is self-limited with fever and headache lasting two days. There is generally no direction for the diagnosis of yellow fever, except in epidemiological surveys, outbreaks and epidemics.
> **b) Moderate form:** The patient has, for two to four days, signs and symptoms of fever, headache, myalgia and arthralgia, conjunctival congestion, nausea, asthenia and some hemorrhagic phenomena such as epistaxis. There may be subtlety. This form, like the mild form, evolves without complications or sequelae.
> **c) Serious form:** In severe cases, after 5 to 6 days of incubation period, the onset of symptoms is abrupt and lasts for 4-5 days with high fever, accompanied by the Faget's sign (decreased pulse), severe headache, severe myalgia, jaundice, epistaxis, epigastric pain and hematemesis and melena (SBI, 2017, p. 6).

In addition to dengue and yellow fever, another arbovirus called Chikungunya, was recognized as an independent entity from dengue in 1950 (Morens and Fauci, 2014), and had its virus, called Chikungunya (CHIKV), *Alphavirus* of the Togaviridae Family, isolated primarily in Tanzania in 1952, due to an outbreak in the region (Aguirre, 2018).

It has a broad clinical aspect, with symptoms that appear four to seven days after infection, and may present in an acute or chronic form. As symptoms, individuals may experience high fever, chills, headache, vomiting, fatigue, back pain, muscle pain and symmetrical arthralgia, which can remain for a long time, considerably impairing the sick individual's quality of life (Castro et al., 2016).

Another febrile illness that is mainly transmitted by A. aegypti is fever by the Zika virus (ZIKV). This, in turn, is an RNA virus (ribonucleic acid virus), belonging to the genus Flavivirus, family *Flaviviridae*. It has three main strains, two from Africa, and one from Asia (Luz et al., 2015). Pinto Junior et al. (2015) points out that there is still no complete knowledge about the clinical manifestations caused by ZIKV. Despite this, the most abundant symptoms identified are, in addition to fever, headache, rash, edema, malaise and joint pain (Vasconcelos, 2015). Recent studies have also shown that Guillain-Barré Syndrome can also be related to previous Zika virus infection (Nóbrega et al., 2018).

Aedes aegypti control strategies

In 1955, the Brazilian government eradicated *A. aegypti* for the first time in history by planning practical actions against the yellow fever vector, with the support of the Pan American Health Organization and the World Health Organization. due to failures in entomological surveillance and intense urban growth, new infestations have been occurring (Braga and Valle, 2007; Marchioro, 2016). In this way, the development of preventive measures aimed at reducing the spread of mosquitoes, as well as effective control strategies, have been carried out constantly (Ragonha and Nowak, 2018).

Among the main methods for the control of *A. aegypti*, the mechanical, chemical and biological methods stand out. Mechanical control consists of eliminating mosquito breeding sites, properly managing materials, removing possible oviposition sites and imposing mechanical barriers so that the insect does not complete its life cycle (General Coordination of Health Surveillance, 2011).

Chemical control, as the name suggests, consists of the use of insecticides to control the adult form of the vector, which can only be used when there are confirmed cases (General Coordination of Health Surveillance, 2011). Among the nine products recommended by the WHOPES (Word Health Organization Pesticide Evaluation Scheme) for the chemical control of *A. aegypti*, five are neurotoxic, with four organophosphates and one spinosyn (Valle et al., 2015), as shown in Table 1.

Table 1. Products against larvae.

Active principle	**Action**	**Class**
Clorpirifos	Neurotoxic	organophosphate
Fention	Neurotoxic	organophosphate
Pirimifós-metil	Neurotoxic	organophosphate
Temephos	Neurotoxic	organophosphate
Spinosad	Neurotoxic	spinosyn
Bacillus thuringiensis israelensis	Lysis of intestinal ephitelium	biolarvicide
Diflubenzuron	development regulator	Chitin synthesis inhibitor
Novaluron	development regulator	Chitin synthesis inhibitor
Piriproxifen	development regulator	Juvenile hormone analog

Source: Valle et al. (2015).

Although chemical control is considered an effective method, it has been the subject of discussions due to its disadvantages, including environmental imbalance, selection of resistant insects and damage to human health (Lopes et al., 2017). In this sense, biological control has stood out in management programs, as it uses predators or pathogens, to reduce the vector population (Zara et al., 2016).

Among the various larvicides used as *A. aegypti* controllers, those of fungicidal origin (Gomes et al., 2015), plant extracts (Porto et al., 2017), seaweed (Salvador-Neto et al., 2016), essential oils (Ferreira et al., 2015), and different species of bacteria, as they have a great capacity for multiplication and dispersion in the environment (Pontes, 2018).

In this context, *Bacillus thuringiensis* (Bt) stands out, which, for producing highly toxic proteins for the larvae of this mosquito, has become one of the most important entomopathogenic species from a scientific, industrial point of view (Costa, 2009), and a commercial product most widespread in the national and international market for use in biological control (Blas-Cerdán et al., 2017).

The discovery of *Bacillus thuringiensis*

According to Nascimento (2003), Bt entomopathogenic bacteria were discovered and exploited commercially since the 20th century. In 1902 in Japan, Shigetane Ishiwata first described a spore-producing bacterium, responsible for the mortality of *Bombyx mori* (silkworm), calling it *Bacillus sotto*, this being the first mention of insect diseases caused by this type of bacteria (Silveira, 2008).

In 1911, Berliner redescribed the same bacterium isolated from caterpillars of the *Anagasta kuhniella* flour moth (Lepidoptera: Pyralidae), and, in 1915, started to call it *Bacillus thuringiensis* in honor of the region of Thuringia, Germany, where the caterpillars were collected (Whiteley and Schinepf, 1986; Dias, 1992; Glare and O'Callagham, 2000).

Berliner mentioned, in 1915, the presence of crystals in Bt spores, however, their activity was only discovered later (Polanczyk and Alves, 2003). From 1920, French farmers began to use Bt as a pesticide (UCSD, 2008). Ramos (2008) points out that the first insecticidal product used for agriculture, formulated and marketed from Bt spores, was developed in France from 1938. It was called Sporeine and used to control *Plodia interpunctella* (Lepidoptera: Pyralidae), popularly known as caterpillars of the flour moth.

In 1953, Hannay verified that the pathogenicity of Bt was determined by the protein inclusions formed during sporulation, a hypothesis validated by Angus in 1968 (Burges, 2001).

With the development of flagellar serology in 1962, there was a great advance in the systematics and classification of entomopathogenic bacilli for the subspecies *Bacillus thuringiensis* (Barjac and Franchon, 1990). This method of classification emerged as a solution to the great problems faced by scientists, due to the large number of varieties of bacilli that arose over time. It consists of testing the strain, hitherto unknown, in the presence of the antibodies of each bacterial lineage already known, allowing the specific differentiation of each one, based on cultural and biochemical characteristics (Medeiros, 2004).

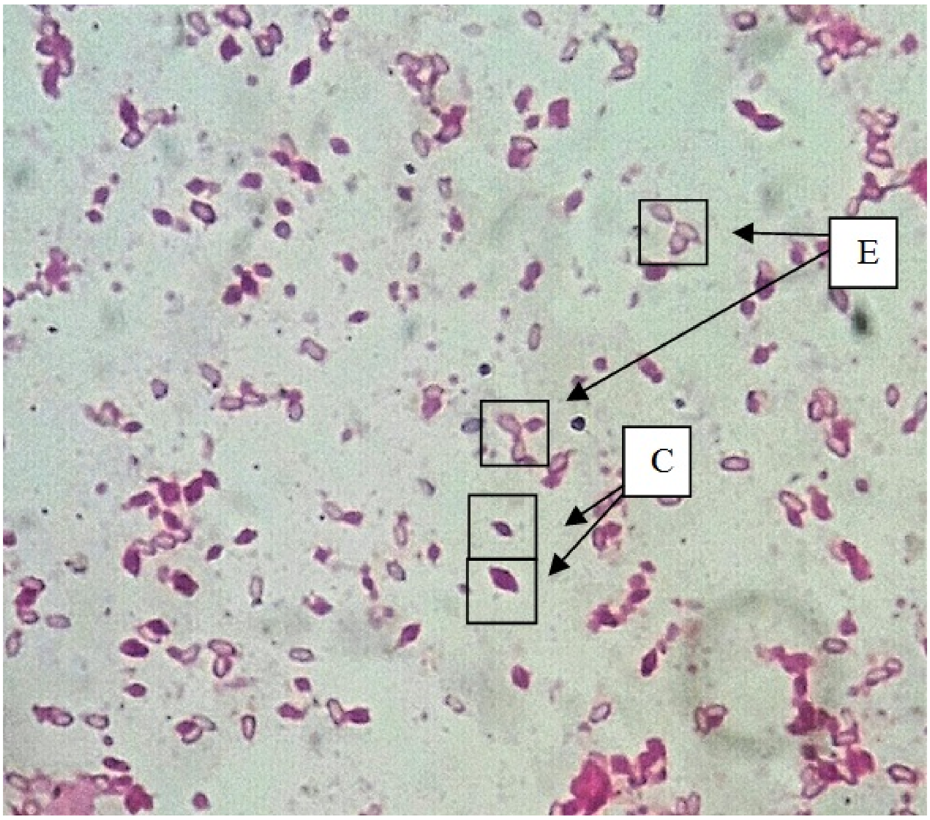

Figura 2. Photomicrograph of *Bacillus* spp. C - bipiramidal crystal; E - spore. Fuchsin staining. 1.000x magnification. Observed with immersion oil.

Bacillus thuringiensis

Bacillus thuringiensis Berliner (Bt) are bacteria belonging to the Bracillacea family. Due to the shared genetic similarities, Bt is included in a complex with other species of bacilli, *B. cereus, B. anthracis, B. mycoids, B. pseudomycoids, B. cytoxicius* and *B. weihenstephanensis* (Sanahuja et al., 2011; Rabinovitch and Vivoni, 2016).

Like other bacteria, it can remain latent in the form of endospores under adverse conditions (Bobrowski et al., 2003). There are many strains of Bt, and, during the sporulation phase, each of them produces one or more toxins concentrated in the form of protein crystals (Figure 2) (Galzer and Azevedo Filho, 2016; Garcia and Santos, 2016; Marchioro, 2016).

Due to its ecology, Bt is one of the most promising bacteria in biological control, with products based on this bacterium corresponding to approximately 90% of the world market for bioinsecticides (Vilas-Bôas et al., 2007), with about 50,000 strains known (Silveira, 2008).

It has the shape of a gram-positive rod, aerobically or optionally anaerobic, with a vegetative cell of 1.0 to 1.2 µm wide by 3.0 to 5.0 µm long, being found naturally in soils, forming spores. In addition to the soil, Bt isolation programs have found strains of this microorganism in living or dead insects, stored grains, phylloplane of plant species, as well as in water samples from rivers and lakes (Capalbo et al., 2005).

These toxins have shown entomopathogenic activity against insects of the orders Lepidoptera, Coleoptera, Hymenoptera and Diptera, in addition to nematodes, mites and protozoa (Schnepf et al., 1998; Praça et al., 2004). For this reason, it has been evaluated for the biological control of A. aegypti larvae, due to the advantages provided by the indiscriminate use of chemical insecticides over the years (Polanczyk et al., 2003).

Bacillus thuringiensis toxins

Bacillus thuringiensis produces, during sporulation in the mother cell, a parasporal crystal containing toxic proteins. These are active for several orders of insects and highly specific to their targets, however, they are harmless to humans, vertebrates and plants, in addition to being completely biodegradable (Pinto and Fiuza, 2008).

El-Bendary (2006) quotes that Rowe et al. (1987) and WHO (1999) describe nine toxins present in Bt lines, as shown in Table 1.

Table 1. Toxins described in *Bacillus thuringiensis* strains.

Toxin	Comment
α-exotoxin	Phosphotypase C
β-exotocin	Thermostable exotoxin
γ-exotoxin	Toxic to insects of the suborder Symphyta (saw flies)
δ-endotoxin	Parasporal Crystal Protein
Lice factor exotoxin	Active only against lice
Rat factor exotoxin	Toxic against mice and *Lepidoptera*
Vip3A	Vegetative insecticidal protein Bt
Enterotoxin	Produced by vegetative cells

Source: Adapted from El-Bendary (2006).

Most Bt crystals are made up of Cry proteins, and they are encoded by genes usually located on plasmids, and, less frequently, on the bacterial chromosome (Galzer and

Azevedo Filho, 2016). Currently more than 800 cry genes are described and, constantly, new genes are discovered.

In addition to Cry proteins, several other toxins can be produced by Bt, such as, Cyt protein, α-exotoxin, β-exotoxin, hemolysins, enterotoxins, chitinases, proteases, phospholipases and vegetative insecticidal proteins (VIPs) (Lima, 2010; Corrêa, 2007; Galzer and Azevedo Filho, 2016).

The Cyt protein is produced mainly by the Israeli variety of Bt. It is a non-specific cytolysin, which accumulates in the crystals together with the δ-endotoxins typical of this variety. It has a molecular weight of 28 kDa (Costa et al., 2010).

A-exotoxin, also known as phospholipase C, is an enzyme with cytolytic activity, which acts on cell membrane phospholipids. It is highly toxic to some insects, either by oral or intrahemoceleic administration (Hansen and Salamitou, 2000).

B-exotocin (thuringiensin) is thermostable, acting by inhibiting nucleases, inhibiting RNA synthesis in cells and can cause lesions in tissues of mice and chickens, causing mutations in mammalian physiological systems, making their use controlled or even prohibited in some countries (Horta et al., 2017).

Chitinases and proteases are in the group of exoenzymes produced by Bt that play an important role in pathogenicity to insects (Lima, 2010). When released by the bacteria, they rupture the peritrophic membrane in the midgut, favoring the access of δ-endotoxins to the epithelium (Reddy et al., 1998; Sampson and Gooday, 1998).

Vegetative insecticidal proteins (VIPs) represent one of the greatest discoveries regarding toxins with insecticidal capacity (Fang et al., 2007). Unlike Cry proteins, which unite in insoluble crystals within the mother cell, VIPs are secreted as soluble proteins by some Bt strains in their vegetative growth phase, which limits their application only in the field (Arora et al., 2003). They are produced in the initial stages of the growth of bacteria in culture, and therefore, as they are obtained in advance, both the supernatant and the mixture of spores and crystals obtained after the cultivation of Bt can be used (Monnerat and Bravo, 2000; Soberón and Bravo, 2001).

Control of *A. aegypti* by *Bacillus thuringiensis*

Few species of Bt have priority in synthesizing active protoxins against insects. Among them, *B. thuringiensis* subsp. *israelensis* (Bti) that acts only on the larvae (Nascimento, 2003). Several studies have demonstrated the efficiency of this bacterium in controlling the larvae of A. aegypti. Silva (2017) tested 553 strains of Bt in his work, of which 37 showed pathogenicity against the larvae. Of these strains, 12 caused 100% larval mortality within 24 hours, and 25 reached 100% death within 48 hours.

Dias (2016), identified 31 strains of *B. thuringiensis* subsp. *israelensis*, all of which showed toxicity to third instar larvae of *A. aegypti*. Katak (2015) also obtained positive results regarding the isolation of Bacillus spp. against *A. aegypti* larvae. Of the 41 strains tested, 10 showed larvicidal activity above 50%. In the first 24 hours, only one strain had a mortality rate greater than 50%. And after 48 hours, seven strains had a mortality rate greater than 50%.

Lobo et al. (2018), also performed larvicide tests and obtained relevant results. In this study, 300 Bt isolates were tested and 12 of them caused mortality in *A. aegypti* larvae. Three of the 12 isolates caused 100% mortality within 24 hours. After 48 hours, four isolates caused a mortality rate of 90%, two provided more than 80% mortality, two caused 76.6% mortality and one reached a mortality rate of 66.6%.

Mode of action of *Bacillus thuringiensis* toxins

The specificity of Bti for culicid larvae is related to the presence of toxins that form in the bacteria during the sporulation phase. When these are ingested by the larvae, in alkaline conditions in the intestine, they are broken by the action of proteases, releasing toxic fractions. These bind to specific receptors on the epithelial membrane (Gill, 1995; Yousten, 1996).

According to Beltrão (2006), it is necessary that a series of events happen for the toxic activity of Bt to occur, as indicated in figure 3. Knowles and Ellar (1987); Aronson and Shai (2001) clarify that after ingestion, the proteins that are insoluble and attached to each other forming a crystal, are solubilized at an alkaline pH close to 10 of the mesenteric of most target insects, the first stage of Bti selectivity.

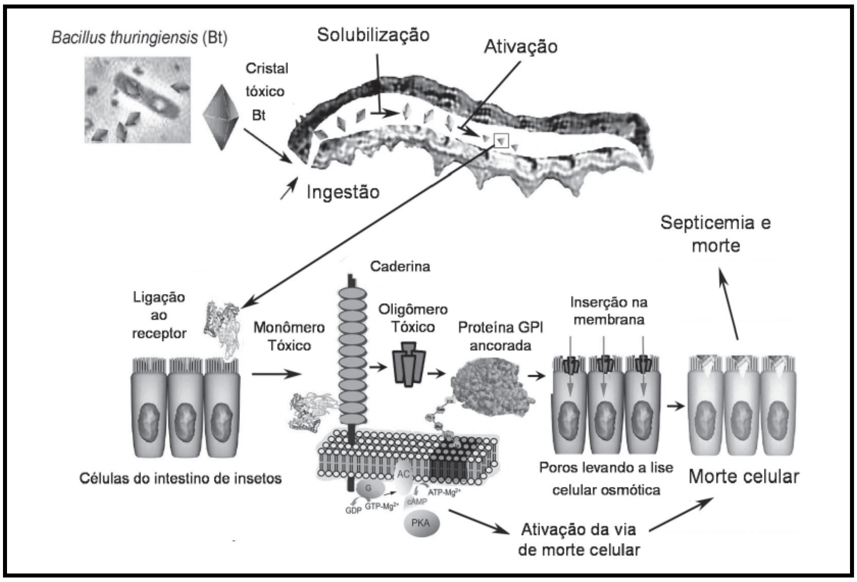

Figure 3. Mode of action of *Bacillus thuringiensis* in insects. Source: Adapted from Horta et al. (2017).

Later, protoxins are released in the larvae intestine, being converted into toxic polypeptides due to the proteolytic action of digestive enzymes. These toxins already activated cross the peritrophic membrane, binding to specific receptors of the apical membrane of columnar cells of the midgut, interfering in the ion gradient and osmotic balance of the apical membrane, increasing its permeability through the formation of pores. The consequence of these events is cell lysis, characterized by the disruption and disintegration of cells (Beltrão, 2006).

Recently, a second model of Bt action has been proven. In this case, protoxin, which was previously believed to be inactive and dependent on cleavage to act, may actually be more potent against resistant insects than its activated toxic fraction (Tabashnik et al., 2015).

This second model points out that there is no need to convert the protoxin into an activated toxin in order for it to be toxic, given that the target receptors are able to bind to the protoxin (Fabrick and Tabashnik, 2007). However, as this is a recent study, much remains to be said about the method, as well as its advantages and disadvantages regarding the classic model of action of Bt toxins.

In vitro experiments with *Pectinophora gossypiella* showed results that indicated that both the protoxin and the activated Cry1Ac toxin bound to fragments of cadherin, which consists of a key protein of the insect's medium intestine receptor (Fabrick and Tabashnik, 2007).

Gómez et al. (2014) cites another in vitro experiment, carried out with Manduca sexta, which demonstrated that both the protoxin and the activated Cry1Ab toxin bound to the same cadherin fragment, so that the protoxin showed a slightly lower binding affinity with respect to activated toxin.

Bacillus thuringiensis in the current market

Although Bt has been used since 1960, only in 1977 after the discovery of *Bacillus thuringiensis* serovariety *israelensis* (Bti), this entomopathogen started to be used as a larvicide for diptera (Beltrão, 2006) being effective for species of the families Culicidae and Simuliidae (Delécluse et al., 2000).

As Monnerat points out; Bravo (2000), several laboratories around the world constantly seek to obtain new Bt isolates that produce new toxins or that are more adapted to local conditions, so that they can be used more efficiently, due to insect resistance. Currently, they represent 80 to 90% of the market for biological agents for mosquito control worldwide (Ootani et al., 2011).

Several Bti-based products are currently being marketed worldwide, as described in Table 2.

Table 2. Products based on *Bacillus thuringiensis* svar. *israelensis* available on the international market for use in culicidae and simulidae control programs.

Commercial name	Formulation	Manufacturer
Aquabac®	Primary powder	Beckar Microbial/USA
Bactimos WP®	Wettable powder	Valent BioSciences/USA
Bactimos PP®	Primary powder	Valent BioSciences/USA
Bactimos G®	Granules	Valent BioSciences/USA
Ice Cubes®	Ice granules	Icybac GMBH/Phoenix/Alemanha
Teknar Hp-D®	Fluid concentrate	Thermo Trilogy/USA
Teknar TC®	Technical powder	Thermo Trilogy/USA
Teknar G®	Granules	Thermo Trilogy/USA
VectoBac 12AS®	Fluid concentrate	Valent BioSciences/USA
VectoBac TP®	Technical powder	Valent BioSciences/USA
VectoBac WDG®	Water-dispersible granules	Valent BioSciences/USA
VectoBac DT®	Tablets	Valent BioSciences/USA
Bactecide®	Water-dispersible granules	BioTech International Ltd/India
Culinex Tab plus®	Tablets	Valent BioSciences/USA
BioTouch®	Fluid concentrate	Zohar Dalia/ Israel

Source: Beltrão (2006)

In Brazil, Bti-based products have acquired special importance, as this biolarvicide has been integrated to combat the vector of dengue (*A. aegypti* Control Program - PCA), conducted in hundreds of municipalities affected by the disease. Several breeding sites of *A. aegypti* received treatment based on this microorganism (Beltrão, 2006).

The use of these bacteria for the biological control of mosquito larvae, has stood out among the various strategies of pest management programs, having various formulations and being used against several species of mosquitoes. These products, generally have a price slightly higher than the products used, however, they are competitive when considering the social and environmental impacts of non-selective insecticides in aquatic systems (Vilarinhos et al., 1998; Polanczyk et al., 2003).

Final considerations

Bacillus thuringiensis represents an efficient alternative for the control of *Aedes aegypti*. The advantages of using bacteria as a source of control are significantly considerable with regard to efficiency, environmental impacts and ease of production and commercialization.

It is necessary, however, that together with new control technologies, efficient forms of awareness are developed for the population, since the vector A. aegypti grows easily in the most varied places, in addition to being highly resistant to environmental characteristics. Only a joint action by science and conscience can eliminate this public health problem in Brazil and worldwide.

Conflict of interest

The authors declare that they have no conflicts of interest.

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