



Biolarvicide Potency of *Bacillus thuringiensis* against Mosquito Larvae and other Insects

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Aims: The aim of the study is to review the mechanism of larvicide bacteria *Bacillus thuringiensis* (Bt) Against mosquito larvae and other insects.

Discussion: characteristics of mosquito larvae is unique and different from any other stage of insect. One of its vulnerabilities is due to its eating habits and this is where Bt toxin play an important role as environmental friendly biopesticide. There are several toxin genes of Bt, namely crystal (*cry*) genes, cytolytic (*cyt*) genes, Vegetative insecticidal protein (*vip*) genes, Secreted insecticidal protein (*sip*) gene, *bin-like* and *ETX_MTX2*-family proteins. These arrays of delta-endotoxins possess toxic properties and can be used as biopesticides and effective vector control against tropical disease such as dengue fever.

Conclusion: Bt toxin is a biological insecticide that acts as a gut toxin to kill insect larvae. Bt is a naturally occurring bacterium that produces spores that contain crystalline inclusions with proteins that are toxic to insects, especially those which responsible for transmitting vector borne disease. Several toxin genes of Bt encode insecticidal proteins that target agricultural and forestry pests which is sustainable and environmental friendly.

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Keywords: Vector control; dengue; fever; tropical disease; biopesticides; cry; cyt; vip; sip; toxin.

1. INTRODUCTION

Morbidity and mortality due to mosquito borne viral infections (Rudolph et al., 2014) such as dengue and chikungunya are gigantic. In 2024, more than 14 million dengue cases and 10 000+ dengue-related deaths were reported globally (European Centre for Disease Prevention and Control, 2025). Most cases were reported from the WHO PAHO in the region of the tropical (WHO PAHO, 2025). While for chikungunya, in 2024 alone, there were 620 000 cases and 213 deaths were detected through epidemic intelligence activities from countries in the Americas (15 published studies or reports), Asia (6 studies or reports), Africa (1 study or report) and Europe (1 study or report) [<https://www.ecdc.europa.eu/en/chikungunya-monthly>].

Nowadays, many infectious diseases were primarily controlled through environmental interventions, one of the most promising is via vector control. In case of dengue and chikungunya, vector control is a key measure to prevent the spread and transmission of dengue and chikungunya (Siagian, 2023; Wilson et al., 2020). Vector control aims to proactively prevent or at least minimize the number of times a vulnerable individual comes into contact with a vector that conveys the seed of the disease (Siagian, 2023; Chen et al., 2016; Eisen et al., 2009; Schorderet-Weber et al., 2017). Principally, it should incorporate a simultaneous act of interventions in the physical environment (Buhler et al., 2019) combined with chemical-insecticide application (Jobe et al., 2023), and also biological agent employment (Siagian, 2023; Thomas, 2018). All of these type of interventions aim to reduce the number of mosquitoes that transmit these diseases. Out of those three interventions, the biological intervention by means of production of microorganisms, mostly pathogenic bacteria, or predatory species or entomopathogenic fungi (Kamel et al., 2024) for use as a biological control agent especially against disease causing insects (Huang et al., 2017). This approach is much easier and costs-effective than the production of chemical insecticides (Colmenarez et al., 2024) which may result to the insects' resistance (van den Berg et al., 2021) and pollution to the environment (Ahmad et al., 2024).

The *Aedes* mosquito is the primary carrier of the dengue virus (Schaefer, 2024). The life cycle of the *Aedes* mosquito, a vector for the dengue virus, is closely related to its role in transmitting disease (Cruz et al., 2023) which relies on its vectorial capacity. The life cycle of a mosquito has four stages: egg, larva, pupa, and adult. All mosquitoes shows to these stages, but different species may prefer different habitats. In a more specific context, the habitat and favorite breeding places of *Aedes* mosquitoes includes relatively clean water in natural or artificial containers (Ferede et al., 2024), such as ponds, plants that hold water or tree holes, flower pots, bottles, cans, non-mounted tires, and clogged gutters (Knoblauch et al., 2024); and in conjunction with its life cycle, except for the adult, all remaining three stages are inhabitant of aquatic milieu (Arévalo-Cortés et al., 2022) as temporary breeder (Glogoza et al., 2000). The good understanding of its life cycle become the pivotal entry point for preventing transmission.

Mosquito larvae, also known as "wigglers", actually are the most vulnerable stage of a mosquito's life cycle; they are prone to their natural predators, oils (because when applied to the water surface, oil creates a barrier that prevents them from accessing oxygen, which they need to breathe through a specialized tube called a siphon; essentially, they suffocate due to their inability to break through the oil film to reach the air at the water surface) and also to certain bacteria. In other words, they are susceptible to biological and chemical larvicides and making them the target of larvicides is quite easy theoretically. Its life cycle continuation can be stop by killing mosquito larvae and pupae before they can grow into adult mosquitoes. The aim of the study is to revisited the mechanism of larvicide bacteria Bt against mosquito larvae and other insects.

2. CHARACTERISTICS OF MOSQUITO LARVAE

The life cycle of a typical mosquito actually has four stages, namely egg, larvae, pupae, and adult (Hawkes & Hopkins, 2022). All types of mosquitoes undergo these stages, but habitats preference varied between species. Mosquito larva (plural- larvae), called "wigglers," are aquatic creatures, can be described as follows: they have three body regions: head, thorax, and abdomen. The head of mosquito larvae is large

and sclerotized (composed of a hardened exoskeleton). Thoracic segments that are strongly dilated compared to the wormlike abdominal ones with apods (no legs), as with all diptera (Martins et al., 2023, Hu, et al., 2020).

Typically, they hang just right below the water surface for respiration (Lee et al., 2017), breathing air through some kind of tubes, a snorkel-like breathing siphon at the distal tip of their abdomen to establish breathe. Larva live entirely in the water and come to the surface to breathe. Larva shed (molt) their skin four times, growing larger after each molt. The periods between molting are called instars. The larval stage lasts between 4 and 14 days. The length of the larval stage depends on the species, water temperature, and food availability (Martins et al., 2023; Lee et al., 2017). This is the most vulnerable stage of the mosquito lifecycle to mosquito control efforts because it is easy to find and destroy in standing water.

One of its vulnerability is its eating habits. Mosquito larvae eat almost constantly until they exit the larval stage- harvest their nutrition by dining on their organic surrounding detritus which can consist of algae, bacteria, protozoa, and other microorganisms that are found in water. It also eats plant debris, insect exuviate and crustaceans. Mosquito larvae filter feed on microorganisms near the water's surface. Mosquito larvae scrape biofilms from rocks and underwater vegetation (Hawkes & Hopkins, 2022). This is when larvicide bacteria come into its potency.

3. *Bacillus thuringiensis*: ITS MICROBIOLOGY AND EFFECTS

Bacillus thuringiensis (Bt) is a ubiquitous Gram-positive, spore-forming bacterium (Sun et al., 2021; sanahuja et al., 2011) that forms a unique parasporal crystal the time of their stationary phase in its growth cycle (Unzue et al., 2022). Their existence in the nature seems to be indigenous to various types of environments, worldwide (Arrieta & Espinoza, 2006; Damgaard, 2000; Assaedi et al., 2011) even considered as environmental pathogens with its unique host specificity which has developed as part of its adaptation to human generated ecological niches (Argôlo-Filho & Loguercio, 2013). Isolation typically involves heat treatment to select for spores (Wen et al., 2022), sometimes with an acetate enrichment step (Rivero & Cerdá-Olmedo, 1987) or alternatively using antibiotic

selection (Ahmed et al., 2024). The diversity in flagellar (H-antigen) agglutination reactions (Xu & Côté, 2008) is one indication of the immeasurable genetic diversity among Bt isolates.

The astonishing diversity of Bt strains and toxins is due at least in part to a high degree of genetic plasticity and mobile genetic elements (Gillis et al., 2018). Most Bt toxin genes appear to be settled in plasmids (Guerrero et al., 2024), on numerous occasion as parts of composite structures that include mobile genetic elements (Hu et al., 2022). Great quantities of Cry gene-containing plasmids appear to be conjugative plasmid (Hu et al., 2022; Guerrero et al., 2024; Gillis et al., 2018).

This Bt was initially marked as an insect pathogen (Ruiu, 2015), and its insecticidal activity was ascribed mostly or completely (depending on the species of insect) to the parasporal crystals (Unzue et al., 2022). Bt has developed an enchanting array of molecular mechanisms to manufacture colossal amounts of biopesticidal toxins (Kumar et al., 2021; Crickmore et al., 2020) during the stationary phase of growth (Palma et al., 2014;). The toxin genes of Bt are crystal (Cry) genes (Crickmore et al., 2020; Peng et al., 2019), cytolytic (Cyt) genes (Guerchicoff et al., 2021; Crickmore et al., 2020) Vegetative insecticidal protein (Vip) genes (Gupta et al., 2021; Crickmore et al., 2020), Secreted insecticidal protein (Sip) gene (Shen et al., 2021; Crickmore et al., 2020) Bin-like and ETX_MTX2-family proteins (Crickmore et al., 2020; Palma et al., 2014). These genes encode insecticidal proteins that target agricultural and forestry pests which is sustainable and environmental friendly (Ragasruthi et al., 2024; Gupta et al., 2021; Chattopadhyay & Banerjee, 2018). Its ecological values are enormous, in addition of being an environmentally-friendly biopesticide (Ragasruthi et al., 2024; Gupta et al., 2021; Chattopadhyay & Banerjee, 2018) and a potential candidate of safe bio fertilizer (Gomis-Cebolla & Berry, 2023), It can also help biodegrade pollutants (organic or inorganic) in the environment (Wu et al., 2023).

4. ITS TOXINS AND THEIR BIOCIDAL ACTIVITY

Bt toxins are proteins produced by the bacterium that are toxic to insects, nematodes, and some human cancer cells. These toxins are used as biopesticides to control insect pests.

Crystal (Cry and Cyt) proteins. Cry and Cyt protein are different, but share the same action, because these two widely known as pore-forming toxins which work in the stomach of an insect (Soberón et al., 2010). These proteins produced during sporulation (Xu et al., 2014) and are the most common type of Bt toxin (Palma et al., 2014). The individual Cry and Cyt proteins actually show weak toxicity to mosquito larvae, compared to the high toxic effect displayed by the whole combined crystal, which results from the synergism among these proteins (Silva-Filha et al., 2021). These two work together to kill mosquito larvae by forming pores in the midgut cells of the larvae (Silva-Filha et al., 2021; Cancino-Rodezno et al., 2012). Its mechanism of action is as follow: Mosquito larvae ingest the Cry and Cyt toxins, which are contained in crystal inclusions. The alkaline pH of the midgut dissolves the crystals (Koller et al., 1992). Midgut proteases activate the protoxins where the structural changes that trigger receptor binding upon proteolytic activation of these insecticidal protein (Infante et al., 2024). The Cry and Cyt toxins bind to receptors and insert into the midgut cell membrane (Bravo et al., 2007). The toxins form pores (Soberón et al., 2010) that disrupt the cell's ion homeostasis and kill the cell. The combination of cry and cyt genes allows for high toxic activity, causing immediate larval death within or less than 24 hours after initial exposure (Soares-da-Silva et al., 2017). The ingestion of the crystals is important for the mode of action since it was observed that larvae treated with soluble toxins did not display mortality (Lopez-Molina et al., 2021) and this is perfectly facilitated by the voracious nature of the larval stage of the mosquito (Hawkes & Hopkins, 2022).

Vegetative insecticidal proteins (Vips). These proteins are secreted during vegetative growth and are toxic to lepidopteran, coleopteran, and some hemipteran pests (Gupta et al., 2021; Palma et al., 2014). The mechanism of action of Vip toxins involves binding to receptors in the midgut of an insect, forming pores, and killing the insect (Infante et al., 2024). Vip1 and Vip2 are binary toxins which are specific to coleopterans and hemipterans (Gupta et al., 2021; Geng et al., 2019). Vip1 binds to receptors in the midgut membrane while Vip2 enters the cell through endocytosis and acts against actin in its target cells- precisely by preventing the formation of microfilaments through ADP-ribose catalytic transfer from NAD to actin and disintegration of insect's cytoskeleton, resulting in the death of

target insect (Sellami et al., 2016). On the other hand, Vip3 toxins are activated by proteolysis in the insect midgut (Nimsanor et al., 2020). The activated toxins bind to receptors, forming pores in the midgut cells. The mechanism of action of Vip3 toxins is similar to the Cry toxin-like protease activation (Byrne et al., 2020) where toxicity is likely to result from pore formation leading to cell necrosis, however, other mechanisms of toxicity have been suggested including the initiation of cell apoptosis via mitochondrial dysfunction and with lysosome play an important role in the action of this toxin (Hou et al., 2020).

Secreted insecticidal protein (Sip). This protein has insecticidal activity against coleopteran pests (Shen et al., 2021; Crickmore et al., 2020). Structural insight into Bt Sip1Ab reveals its similarity to ETX_MTX2 family beta-pore-forming toxin (Chen et al., 2023). It likely functions by binding to receptors in the midgut of insects and disrupting the membrane. This disrupts the function of the midgut and allows gut contents to leak out, which kills the insect.

Bin-like and ETX_MTX2-family proteins. These proteins are produced by some Bt strains and share amino acid similarities with mosquitocidal binary (Bin) and Mtx2 toxins. Binary (BinAB) toxin is primarily responsible for the larvicidal effect of the bacterium. BinAB is a single-receptor-specific toxin which act like a stomach poison and is effective against larvae of *Culex* and *Anopheles*, but not against *Aedes aegypti*. (Sharma & Kumar, 2021; Smith et al., 2004).

One may only conjuncture regarding the ecological contribution to the bacterium of implicating several Cry gene expression systems, which strongly suggest the broader spectrum of insecticidal activity during its active portion (Arsov et al., 2023). Unfortunately, co-expression of multiple toxins is distinctly possible to elevate the host range of a given strain (Malovichko et al., 2019) or of a population exchanging toxin genes (Gonzalez et al., 2019); it was suggested there was plasmid transfer between different Bt strains during growth within an insect which was a non-susceptible larvae (Thomas et al., 2001). Any critical experiments directed towards understanding bacterial toxin gene expression within the gut of a susceptible pest must be encourage to make a better understanding regarding its biopesticidal properties, especially in mechanism of action and also interaction with its insect host.

5. CONCLUSION

Bt toxin is a biological insecticide that acts as a gut toxin to kill insect larvae. Bt is a naturally occurring bacterium that produces spores that contain crystalline inclusions with proteins that are toxic to insects, especially those which responsible for transmitting vector borne disease. There are several toxin genes of *Bacillus thuringiensis*, namely (Bt) are crystal (Cry) genes, cytolytic (Cyt) genes, Vegetative insecticidal protein (Vip) genes, Secreted insecticidal protein (Sip) gene, Bin-like and ETX_MTX2-family proteins. These genes encode insecticidal proteins that target agricultural and forestry pests which is sustainable and environmental friendly.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as large language models (chatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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