

Short Communication

Pathogenecity of *Bacillus thuringiensis* against the dengue vector *Aedes aegypti* larvae

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Accepted 24 October, 2011

Bacillus thuringiensis is ubiquitous, Gram-positive, spore-forming bacteria that act as a larvicide to various Dipteran species due to the toxin it produce. Sporulation process, the production of endotoxins, of the isolated bacterial strain varies in which some isolates sporulate faster while other isolates took some time for sporulation. Thus, the isolate with faster sporulation process were chosen. In determining the LC₅₀, concentration of the bacterial density was based upon the McFarland standard (4 to 0.5×10⁸ cfu/ml) and 1 ml of bacterial culture was added into cups with twenty (20) *Aedes aegypti* larvae and was observed for 24 - 48 h. Positive control exhibits lethality at an early hour of inoculation having the highest larvicidal potency with LC₅₀ value of 0.36 cfu (colony forming units)/ml at 24 h and 0.57 cfu/ml at 48 h. Among the different *B. thuringiensis* isolates tested, toxicity and lethality of the bacterial isolates was only observable after 48 h. Isolates from the garden soil exhibits the highest larvicidal potency with LC₅₀ values of 0.85, 0.97, 0.13, and 0.32 cfu/ml. These results suggested that *B. thuringiensis* isolated from garden soil is promising as larvicide against the target Dengue virus carrying mosquito.

Key words: Larvicidal, sporulation, colony forming units (cfu), *Bacillus thuringiensis*, *Aedes aegypti*.

INTRODUCTION

Mosquitoes are common in tropical and subtropical regions as the vectors by spreading pathogens of many important diseases to humans and animals and have been the subject for control since the early 1940s with chemical insecticides. Thus, after initial success in the control was obtained, the insects developed resistance to chemical insecticides. The failure of chemical insecticides to fully control these insect vectors make it necessary to find other ways of controlling vectors. One such approach is the use of biological control (Obeta and Okafor, 1984). The production of microorganisms, mostly bacteria, for use as a biological control agent especially against disease causing insects is much simpler and costs less than the production of chemical insecticides which may result to the insects' resistance and pollution to the

environment. The bulk production of bacteria can be undertaken in many undeveloped countries in which the diseases borne by some insects are endemic and is causing a lot of lost lives and livelihood (affecting the agricultural products).

B. thuringiensis var *israelensis* (Bti) produces *cry* toxins which acts as a larvicide. *Cry* toxins is activated by the midgut alkaline pH of the mosquito larvae and disrupts the midgut epithelial cells (Davidson and Sweeney, 1983; WHO, 1999). These crystal proteins are protoxins that are proteolytically converted into smaller toxic polypeptides in the insect midguts. Following ingestion by mosquito larvae, the inclusion bodies are solubilized, and the protoxins are converted to toxins (Hofte and Whiteley, 1989). The control of dengue vectors at the larval stage is much easier compared to adults since, the movement of the larvae is limited to its aquatic habitat whereas the adults by and large do not rest on sprayable surfaces and there is greater difficulty in ensuring effective contact

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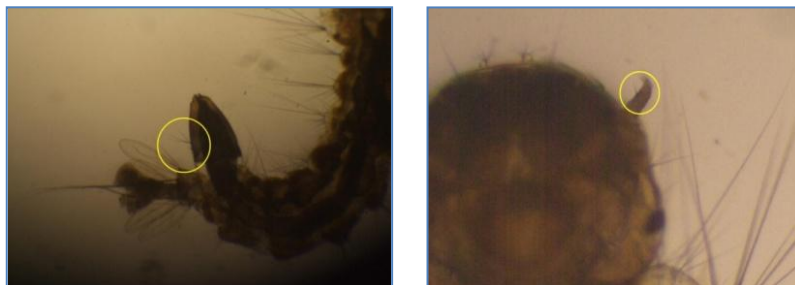


Figure 1. *Aedes aegypti* larvae: (left) presence of three branches of hairs that arise on the same socket on the airtube of the larvae; (right) the presence of only one branch of antennal hair.

of adults with insecticides (Wang and Jaal, 2005).

MATERIALS AND METHODS

Isolation and identification of *B. thuringiensis*

Isolation and identification of the *Bti* strain was conducted according to the method described by Aramideh et al. (2010), Raymundo et al. (1991) and Travers et al. (1987).

Bioassay of *B. thuringiensis* against *A. aegypti* larvae

The mosquito larvae samples were obtained from Barangay Pugan, Tibanga, and Tambo, Iligan City. Classification of *A. aegypti* larvae were based on its significant characteristics, which are unique for the species. A larva was placed in a depression slide with a little amount of water enough for the larva to move and was examined under Leica compound microscope; hence several morphological characteristics were noted such as the presence of hair in the different parts of the body, structure of air tubes, number of hair in the antenna and siphon, etc. (Figure 1).

All strains of *B. thuringiensis* were grown in T3 medium for 48 h at 30°C. In order to determine the LC₅₀, McFarland Standard was used as standard in determining the bacterial density prepared as aforementioned was used.

One ml of bacterial culture with different bacterial density according to the Mc Farland Standard (4-0.25 × 10⁸ cfu/ml) was added into 200 ml cups in triplicate. Forty-eight hours later the numbers of dead larvae were recorded and the LC₅₀ (lethal concentration necessary to kill 50% of larvae) was calculated by Probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

B. thuringiensis is normally found in the soil environment. The ubiquity of *B. thuringiensis* in soil supports the hypothesis several authors suggesting that this is the normal environment of *B. thuringiensis*. In this study, *B. thuringiensis* were isolated from three soil samples (vermicast soil, garden soil, and corn crop soil) and was identified according to the Bergey's Manual of Identification of Bacteria applying the selection method by Travers et al. (1987).

Figure 2 shows the percentage and probit mortality,

and the LC₅₀ of the *Bti* (the positive control) and the ten isolated strains tested against the identified larvae of *A. aegypti*. Thus, the positive bacterial strain showed highest larvicidal potency with LC₅₀ value of 0.36 cfu/ml at 24 h and 0.57 cfu/ml at 48 h. Isolated *Bti* from the vermicast soil, particularly isolate 1C, showed the highest larvicidal potency among the group with LC₅₀ value of 2.66 cfu/ml at 24 h and 0.74 cfu/ml at 48 h. Other isolates also exhibit near larvicidal potency at 48 h. It is during at this time are the bacterial cells undergoes the sporulation process producing the endospores together with the toxic crystal proteins.

Isolates from the corn field (2A, 2B, 2D) area were also tested and its lethal concentration was determined according to the tests stated above. Thus from this isolates, the highest larvicidal potency at 24 h is only 2.75 cfu/ml. A significantly high difference between the positive control.

Isolates from the garden soil (3A, 3B, 3S, 3D) exhibits larvicidal potency with the isolate 3C having the highest LC₅₀ value of 2.53 cfu/ml at 24 h and 0.13 cfu/ml at 48 h. The lethality of the said isolates which exceeds those of the positive control maybe attributed to the characteristic of the isolates which sporulate at a late period of time.

Some studies involved diversity and ecology of the *B. thuringiensis* and these studies showed that *Bti* is not normally toxic to insect larvae that inhabit the soil environment, such as the worms. But other studies supports that it is toxic to insects that have aerial or water-borne larvae, that is mosquitoes. Thus, the toxicity of the *B. thuringiensis* is mainly because of its crystalline proteins produced during the sporulation stage. These results suggested that *Bti* isolated from soil is promising as larvicide against the target Dengue virus carrying mosquito.

Conclusion

B. thuringiensis acts as a larvicide due to the toxin produced, which is activated by the alkaline midgut of the mosquito larvae and disrupts the midgut epithelia. Thus,

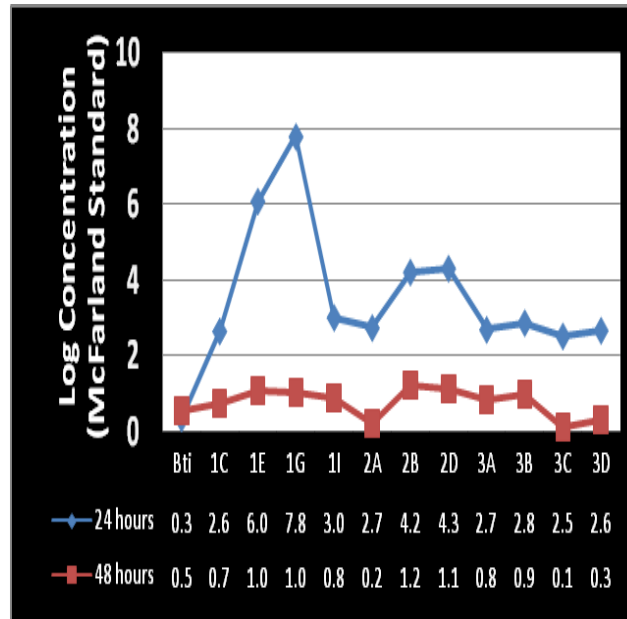


Figure 2. Log Concentration by McFarland Standard of the different isolates tested against *A. aegypti* larvae.

this observation was proven when at an early hour of inoculation, *A. aegypti* larvae were considered dead even at 4 h. With increasing time, toxicity levels also increase, giving it the highest larvicidal potency at 24 h. The same is true at 48 h. However, an isolated *Bti* from garden soil exceeds the said larvicidal potency of the positive control with a LC_{50} value of 0.13 cfu/ml compared to 0.57 cfu/ml of the positive control. The study suggests the screening of more *Bti* to be isolated from different sources of samples. Still further researches are required to produce an alternative to synthetic pesticides. Further investigation is needed to identify the active crystalline protein components of these strains responsible for its activity. The important biological activity of *B. thuringiensis* isolates may be derived from the combined attributes of different *Cry* toxins formed into a proteinaceous body. Determination of their *Cry* gene contents will be useful for the prediction of their toxicity.

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