Abstract

Bacillus thuringiensis, commonly referred to as *B.t*, is renowned as the premier microbial pesticide. Its effectiveness extends to various insect orders, including Lepidoptera, Coleoptera, and Diptera. *B.t* produces diverse crystal proteins, showcasing its larvicidal capabilities against a broad spectrum of insects. It displays specificity in its target, proving effective not only against Nematodes but also against human cancer cells. The recent study focused on evaluating its larvicidal activity against mosquitoes, specifically *Aedes aegypti* and *Anopheles stephensi*, which are responsible for causing diseases such as Malaria, Yellow Fever, Filariasis, and Dengue through their bites. In this study, nine previously isolated and characterized *B.t* strains were selected for assessment. To determine their biotoxicity, a comparative analysis was conducted using a negative control, *Bacillus subtilis*, and a positive control, HD 500. The *B.t* spore dose and protein dose were prepared. Initially, an isolated colony of *B.t* was streaked on LB agar plates and incubated at 37°C for 24 hours. Subsequently, T3 LB agar plates were prepared and incubated at the optimal temperature for another 24 hours. After incubation, isolated colonies were identified, and a single colony was selected using a sterilized inoculating loop. This colony was streaked onto freshly prepared T3 plates and placed in the incubator for 72 hours at temperatures ranging from 34 to 37°C. After three days, the bacterial lawn was scraped off, and the pellet was washed five times. The pellet was then treated with buffer one (KCl and Na_3PO_4 buffer, pH 7.8) and incubated for 40 minutes, followed by two washes. Subsequently, the pellet was treated with the second buffer (urea and CHES buffer, pH 10-11) and incubated for 30 minutes. After two additional washes, the pellet was stored at 4°C and later oven-dried at 70°C for further use. Doses ranging from 100 to 1000 µg/ml were prepared for the experimental bioassay, with triplicate setups. Each setup included ten 3rd instar larvae in 10ml of autoclaved distilled water. The larvicidal effect of varying concentrations of the spore dose was assessed after 48 hours, with larvae that were immobilized and unable to swim being considered deceased. The number of dead larvae was counted, and the LC50 value was determined using the Probit analysis method. For the protein dose, plates were prepared using the same method as described above, with the only difference being the use of a different buffer (Na_2CO_3, DDT, and water buffer, pH 10-11). After two additional washes and centrifugation at 7000rpm for 15 minutes at 4°C, the buffer was introduced to the pellet, which was then placed in a shaking incubator for 3 hours. Following two more washes, the supernatant was collected, and the pH was adjusted to 7-8 using 1N HCl. Out of the nine B.t isolates tested, GCU-DAB-B2.1 exhibited the highest toxicity against Aedes aegypti and Anopheles stephensi larvae, making it the most potent strain among the nine tested.