

Thesis Title Molecular Cloning and Expression of
Mosquito Toxin Gene from *Bacillus*
thuringiensis subsp. *israelensis* in
Escherichia coli and *Bacillus sphaericus*
strains 1593 and 2362.

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ABSTRACT

A 3.7 kb XbaI fragment encoding a 130 kilodalton (kDa) mosquito toxin from a 110 kb plasmid of *Bacillus thuringiensis* subsp. *israelensis* (*B.t.i.*) strain 4Q2-72 was cloned into pUC12 and transformed into *E. coli*. The clone with a recombinant plasmid (designated pBT8) was toxic to *Aedes aegypti* larvae. pBT8 (6.4 kb) was ligated into pBC16 (Tetracycline resistance, Tc^r) and transferred into *E. coli*. The new recombinant plasmid was designated pBT24. pBT24 or its deleted plasmid, pBTC1 which was constructed by deleting SmaI-SalI fragment of pUC12 were transformed by the method of protoplast transformation into *Bacillus sphaericus* strains 1593 and 2362 which were highly toxic to *Anopheles* and *Culex* mosquito larvae but less toxic to *Aedes* larvae. After cell

regeneration on regeneration medium, the Tc^r plasmids from transformants (pBTC1) of both strains of *B. sphaericus* were prepared and analysed. The 3.7 kb XbaI fragments from *E. coli* and *B. sphaericus* transformants were shown to be present by agarose gel electrophoresis and Southern blot hybridization. In addition, *B. sphaericus* transformants produced a 130 kDa mosquitocidal toxin which was detected by Western blot analysis with antibody prepared against *B.t.i.* 130 kDa mosquitocidal toxin. The LC₅₀ of the transformants of strains 1593 and 2362 against *Ae. aegypti* larvae were 5.75×10^2 and 2.71×10^2 cells/ml respectively. This level of toxicity was comparable to the LC₅₀ of *B.t.i.* but much higher than that of *B. sphaericus* 1593 and 2362 (4.7×10^4 cells/ml) against *Ae. aegypti* larvae. The transformants also retained high toxicity against mosquito larvae of *Anopheles* and *Culex*. Finally, the recombinant plasmids were highly stable upon daily subculture for at least 4 weeks in medium without tetracycline.