ABSTRACT

*Bacillus sphaericus* has been identified as a potential alternative to chemical pesticides for mosquito control program. The lethality of *B. sphaericus* to mosquito larvae is mainly due to two proteins (51 and 42 kDa) and they act together as binary toxin.

In the first part of the present study, genes for the binary toxin have been cloned under the control of T7 promoter and expressed in *E. coli* which resulted in high levels of expression of binary toxin under uninduced conditions. The "leaky" regulation of the T7RNA polymerase gene was responsible for the constitutive expression of binary toxins.

In the second part of the study, site-directed mutagenesis was used to generate mutant binary toxins to investigate their mode of action. Alanine residue was selected to substitute some of the amino acids located at the N- and C-terminal regions of the binary toxins and the effects of these changes towards biological activity were assessed. The bioassay results showed that alanine substitutions in 51N-terminal gut binding domain caused loss of biological activity, indicating that these amino acids may be involved in receptor binding. Similarly, alanine substitution in 51C-terminal or 42N-terminal also abolished the biological activity suggesting that these amino acids may be important for interaction between 51 and 42 kDa peptides. Replacement of a single amino acid at the C-terminal of the 42 kDa also abolished the biological activity, suggesting that this amino acid may play an important role in internalization of the toxin complex.
Surprisingly, the biological activity was restored when the two non-toxic mutants of same peptide (one N-terminal and one C-terminal mutants of 51 kDa) were mixed and tested for toxicity against mosquito larvae. This indicates that non-toxic mutant proteins functionally complement each other which resulted in restoration of their function.

In the third part of the study Polymerase Chain Reaction and non-radioactive probes were used to monitor the *B. sphaericus* released in the environment. Digoxigenin based non-radioactive method was applied for the detection of PCR products and its sensitivity was high enough to detect less than ten *B. sphaericus* cells. A simple and inexpensive sample processing procedure has also been developed for direct PCR amplification of *B. sphaericus* DNA from field samples.