Thesis Title A Study of The Expression of *Bacillus sphaericus* Mosquito- larvicidal Toxin Genes in *Escherichia coli*

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Abstract

Chemical insecticides have been valuable in the control of insect vectors of some diseases but their use has also posed certain problems. They often contaminate the environment and tend to harm non-target organism. One of the promising alternatives to avoid such problems is the use of microorganisms as agents to control insect pest.

Bacillus sphaericus (Bs) is one of the biological control agents which produce potent proteins with specific mosquitocidal activities. Highly larvicidal strains of Bs produce a binary toxin composed of the 51- and 42 kDa toxin proteins during sporulation, both of which are required for toxicity. It has been reported that the expression of the 51- and 42 kDa toxin genes of Bs in *Escherichia coli* is quite low. In this study , the Bs toxin genes were put under the control of *lacZ* or T7 promoter with the designed Shine- Dalgano sequence (SD sequence) and start codon , in order to improve the Bs toxin genes expression in *E. coli* . The 2.65 kb

DNA fragment containing the 51 kDa and 42 kDa toxin genes were amplified from plasmid pCC2297-M using the Polymerase Chain Reaction (PCR) . These amplified toxin genes containing the designed SD sequence and the start codon were put under the control of *lacZ* promoter in pUC18 vector to obtain plasmid pUCBs2297, or put under the control of T7 promoter in pT7-5 vector to obtain plasmid pT7-5Bs2297.

In order to detect the expression of the toxin gene in *E. coli* , Immumoblot analysis was used . Rabbit antisera raised against the 51 kDa or the 42 kDa protein were prepared using maltose fusion and purification system.

Western blot analysis revealed that under the control of three different promoters , T7 (pT7-5Bs2297), *lacZ* (pUCBs2297), and original Bs promoter (pCC2297-M) , expression of both 51- and 42 kDa toxin genes under the control of the T7 promoter was the highest , whereas that under the control of *lacZ* is the lowest. Toxicity test showed that at the concentration of 10^7 cells /ml of *E. coli* harboring pT7-5Bs2297, pCC2297-M , or pUCBs2297 were toxic to second instar *Culex quinquefasciatus* at 93.2 $\pm$ 4.7%, 79.8$\pm$ 6.3% and 64.8 $\pm$ 5.9% motality , respectively. This results correlated to the level of toxin proteins expressed in *E. coli* cells.