Thesis Title Construction of Hybrid Endotoxin Genes and Evaluation of Their Mosquito-Larvicidal Activities

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ABSTRACT

The gene encoding 130 kDa-mosquito larvicidal protein of Bacillus thuringiensis var israelensis (B.t.i) crystal, previously cloned in Escherichia coli and named pMU388, has been modified to improve the larvicidal activity by (i) fusion of the toxin gene together, (ii) transposition to pET-3b vector at the position which make this gene under control of a strong promoter of T7 DNA phage. In addition, we present herein a construction of recombinant capable of combining expression of the toxin gene of both B.t.i and Bacillus sphaericus which produce toxin more effective to Culex quinquefasciatus than pMU388.

The recombinant #278 containing fused 130 kDa endotoxin gene produced the 200 kDa polypeptide and was found to give toxicity to Aedes aegypti larvae comparable to the gene product of recombinant pMU388. The recombinant #119 carrying the endotoxin gene inserted in pET-3b vector exhibited toxicity to A.

aegypti larvae at the same level as recombinant pMU388. This result revealed that the T7 promoter of plasmid pET-3b did not improve the larvicidal activity of B.t.i 130 kDa delta-endotoxin. The recombinants habouring hybrid endotoxin gene from B.t.i and B. sphaericus showed toxicity to A. aegypti larvae at the same level as recombinant pMU388 that carry B.t.i endotoxin gene and also toxic to C. quinquefasciatus larvae comparable to recombinant pCC2297-M which habour B. sphaericus toxin gene.