Thesis Title	Gene Cloning and Effect of 20, 68, and 130 kDa Protein from *Bacillus thuringiensis* subsp. *israelensis* Against *Aedes aegypti* Larvae.
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ABSTRACT

The *cryIVB*, *cryIVD* and 20 kDa protein genes, encoding the 130-, 68- and 20 kDa proteins, respectively, of *Bacillus thuringiensis* subsp. *israelensis*, were cloned either alone or in combination into *Bacillus* cloning vector and expressed in a nontoxic strain of *Bacillus thuringiensis* subsp. *israelensis* strain c4Q2-72 and *Bacillus megaterium* strain O-016. The seven recombinant plasmids pBT24 (*cryIVB*), pBTC68 (*cryIVD*), pBTC20 (20 kDa protein gene), pBTC20-68 (20 kDa gene and *cryIVD*), pBTC20-130 (20 kDa gene and *cryIVB*), pBTC68-130 (*cryIVD* and *cryIVB*) and pBTC20-68-130 (20 kDa gene, *cryIVD* and *cryIVB*) could be transferred into *B. megaterium* strain O-016 by protoplast transformation without any gene deletion or alteration. In contrast, only six recombinant plasmids except pBTC20-68-130 could be successfully transferred into *B.t.* c4Q2-72 by electroporation. Transferring of pBTC20-68-130 into *B.t.* c4Q2-72 suffered plasmid deletion of either gene or all genes. The

presence of each gene in each constructed plasmid was confirmed by restriction analysis and Southern blot hybridization. In addition, *B.t.i.* c4Q2-72 and *B. megaterium* O-016 transformants produced a 130- and 68- kDa mosquitocidal toxin which were detected by Western blot analysis with antibody prepared against *B.t.i.* crystal protein. The LC_{50} of both *B.t.i.* c4Q2-72 and *B. megaterium* O-016 transformants with recombinant plasmids assayed against *Aedes aegypti* larvae were performed. It was found that transformants with both *cryIVB* together with *cryIVD* gene had about 10-100 times higher toxicity than those transformants with either gene alone. In *B.t.i.* c4Q2-72 transformants with either *cryIVB* together with 20 kDa protein gene or *cryIVD* together with 20 kDa gene could slightly enhance toxicity against the mosquito larvae when compared with the transformants containing *cryIVB* or *cryIVD* alone. In contrast, the same combination has no different toxicity effect against the mosquito larvae in *B. megaterium* host. For combination with three proteins, it was also shown that *B. megaterium* O-016 (pBTC20-68-130) was toxic to *Aedes aegypti* larvae for at least 100 times higher than the transformants which produced single protein and about 10 times higher than the transformants which produced both CryIVB and CryIVD proteins.