Thesis Title Cloning and Expression of *cryIVB* gene of *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) in Locally Isolated Cyanobacteria

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Date of Graduation 10 August B.E. 2537 (1994)

ABSTRACT

The cyanobacteria are organisms those are capable of oxygenic photosynthesis. They have become an increasing popular model system for many fields of investigation via genetic and molecular biological techniques. Many attempts to manipulate the mosquito-larvicidal genes of both *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) and *B. sphearicus* to be expressed in these group of organisms have proven unsatisfactory. Since all previous studies were done on the reference or laboratory strains, it is more promissing to use the strain(s) which inhabit the same place with mosquito larvae or to use the locally isolated cyanobacteria.

151 isolates of cyanobacteria were obtained from 176 water samples collected from various places in Bangkok and nearby provinces. Five isolates were sent for identification at the Pasteur Institut, France, and found to be *Synechocystis* PCC9001 and PCC9002 and *Synechococcus* PCC9003, PCC9004 and PCC9005. Due to the complication and time-consumption of conventional methods to identify cyanobacteria, identification of bacteria by DNA fingerprinting was applied to differentiate some of those local isolates. DNA from 40 isolates and 3 reference strains from the Pasteur Culture Collection (PCC) were digested with *Pst*I and probed with ^{32}P-labelled M13 bacteriophage DNA to produce characteristic DNA fingerprinting patterns (DFP). 37 DFP were obtained. None of the local isolates gave a similar pattern to those of the reference strains. Six isolates collected from different places gave 3 pairs of identical DFP, and three isolates collected from the same place also gave identical DFP, suggesting that the ones those having identical DFP belonged to the same isolate. 31 isolates gave different patterns suggesting that cyanobacteria were highly heterogeneous.

To introduce the *cryIVB* gene of *Bti* into local cyanobacteria, a shuttle vector was constructed. However transformation by natural uptake or electroporation were not successful.

Using the technique of plasmid mobilization, the broad-host-range plasmid, pKT210, was able to mobilize from *E.coli* (habouring the helper plasmid, pRK2013) into 3 out of 11 isolates of local cyanobacteria. pKT210.388, containing the *cryIVB* gene was mobilized into PCC9002 and CNRS2. The existence of the plasmid in cyanobacteria was demonstrated by Southern blot hybridization and rescue of plasmid in *E.coli*. The 128 kDa protein product from these cyanobacterial transconjugants were demonstrated by immunoblotting assays, although the amounts of products were quite low. However, the results indicated that the *cryIVB* gene of *Bti* was able to be introduced and expressed in locally isolated cyanobacteria.

In an attempt to improve the expression of *cryIVB* gene in local cyanobacteria, pKT210.388.T7.pol which contained T7 promoter upstream to the *cryIVB* gene, T7 RNA polymerase gene and the whole set of its regulatory genes was constructed. In *E.coli* the *cryIVB* gene in this plasmid was shown to be expressed very well after heat induction. The cells were also highly toxic to mosquito larvae. However, expression in cyanobacteria using the same system was not higher than the expression under its own promoter . It was demonstrated by rescue of plasmids in *E.coli* that the *cryIVB* gene in plasmids from cyanobacteria had not been changed within the cyanobacterial cells as the gene was able to express in the rescued *E.coli* to a similar extent as in cells that harboring the original plasmid . The low expression might be due to the low copy number of the broad host range plasmid, pKT210, in cyanobacteria.