

Thesis Title Integration of the 130 kDa Endotoxin
 Gene into the Chromosome of Cyanobacteria
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ABSTRACT

Bacillus thuringiensis variety israelensis (Bti.) is an important biological insecticide in controlling mosquito larvae. However, the major drawback of using Bti. is that it is rapidly adsorbed by soil particles so that the toxin is removed from larval feeding area. Cyanobacteria has been considered as a promising microorganism for producing the toxin of Bti. because this photosynthetic bacteria live in the upper layer of water. Hence, the toxin produced by cyanobacteria may persist longer in the natural habitat of mosquito larvae. In this thesis, the 130 kDa endotoxin gene of Bti. was integrated into the chromosome of cyanobacteria and the expression of the endotoxin gene in cyanobacteria was also determined.

Synechocystis PCC9001, a cyanobacterial strain isolated in Thailand, was first chosen to be the host for integration of 130 kDa endotoxin gene. Two recombinant

plasmids, pCK3-14 and pCK6-1, were constructed as donor DNA molecules by insertion of a Synechocystis PCC9001 DNA fragment flanking neomycin resistance (Neo^{R}) gene and 130 kDa endotoxin gene into Bluescribe vector. Higher amount of 130 kDa protein which could react with anti-130 kDa endotoxin antibody was expressed in E. coli harbouring pCK3-14 than in E. coli harbouring pCK6-1. Neither pCK3-14 nor pCK6-1 could transform Synechocystis PCC9001.

Synechococcus PCC7942 was used as a new host instead. Neo^{R} gene was inserted into the chromosome of Synechococcus PCC7942. Plasmid pIV7D was recovered from the chromosome of kanamycin resistance Synechococcus PCC7942. pIV7D composed of the chromosomal fragment of Synechococcus PCC7942 containing Neo^{R} gene linked to Bluescribe vector. This plasmid can be used as an integrational vector for integration of foreign DNA into Synechococcus PCC7942 chromosome. Synechococcus PCC7942 Ap^{R} was the ampicillin resistance strain of Synechococcus PCC7942 harbouring Bluescribe in the chromosome. pCK6-1 was integrated into Synechococcus PCC7942 Ap^{R} chromosome by genomic integration system based on Bluescribe sequence. The transformants obtained, named Synechococcus PCC7942 CK6-1, were resistant to both ampicillin and kanamycin. The 130 kDa protein expressed by Synechococcus PCC7942 CK6-1 could react with anti-130 kDa endotoxin antibody. However, the amount produced was approximate 10^4 -fold less than in E. coli. The antibiotic

resistance phenotypes and capability of expression of 130 kDa protein related to 130 kDa endotoxin were stable even after growing in the absence of antibiotic for 15 subcultures. However, no mosquito larvicidal effect of Synechococcus PCC7942 CK6-1 was detected at concentration of up to 10^8 cells/ml.