

Thesis Title Effect of Toxin Gene Promoter on Expression of
Chloramphenicol Acetyltransferase Gene (*cat*)
in *Bacillus thuringiensis*

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ABSTRACT

A *Bam*H I-*Pst*I fragment harbouring the promoter region of *cry* IV gene encoding 130 kDa *Bacillus thuringiensis* subsp. *israelensis* mosquitocidal toxin protein from pBTC1 was cloned into the promoter probe plasmid vector, pPL703. The recombinant plasmid pPLC1 was transformed into *Bacillus megaterium* strain 0-016 by protoplast transformation and subsequently was transferred from the transformant to *Bacillus thuringiensis* subsp. *israelensis* strain c4Q2-72 and 4Q2-72 by conjugation-like process with the frequency of 2.1×10^{-7} and 1.7×10^{-7} respectively (number of transconjugant per number of recipient before mating). The presence of cloned promoter region in the constructed plasmid was confirmed by restriction pattern and Southern blot hybridization. The expression level of chloramphenicol acetyltransferase gene (*cat*) was determined

by measuring the specific activity of the enzyme chloramphenicol acetyltransferase (CAT). The *cat* gene product was assayed in crude extracts obtained from lysozyme treated *B. megaterium* strain O-016 transformants and *Bacillus thuringiensis* subsp. *israelensis* transconjugants. Both transformant and transconjugants were grown in NBS medium (nutrient broth supplemented with minerals) and harvested at the various growth phases, namely, mid exponential, T₀, T₂ and T₈. It was shown that the inserted promoter region from *cry IV* gene conferred the post exponential promoter activity according to the growth pattern when compared to those harbouring the relevant plasmid, pPL603, pPL703 and pTF6. There was no significant difference in term of plasmid copy number in all these plasmids in *Bacillus thuringiensis* host. Finally, the recombinant plasmid was stably maintained in *Bacillus megaterium* strain O-016 transformant, but highly stable in *Bacillus thuringiensis* subsp. *israelensis* c4Q2-72 and 4Q2-72 transconjugant upon daily subculture for at least 4 weeks in LB broth medium without any selective pressure.