4136316 MBMG/M : MAJOR: MOLECULAR GENETICS AND GENETIC ENGINEERING; M.Sc (MOLECULAR GENETICS AND GENETIC ENGINEERING)
KEY WORDS : DELTA-ENDOTOXIN, Cry4A, *Bacillus thuringiensis*, DISULPHIDE BOND

WALAIRAT PORNWIROON: INVESTIGATING THE ROLE OF THE PUTATIVE DISULPHIDE BOND WITHIN THE LOOP CONNECTING α4 AND α5 OF THE *Bacillus thuringiensis* Cry4A TOXIN. THESIS ADVISORS: CHANAN ANGSUTHANASOMBAT, Ph.D., CHARTCHAI KRITTANAI, Ph.D., GERD KATZENMEIER, Ph.D. 116 p. ISBN 974-664-423-8

A 3D model of the activated 65-kDa *Bacillus thuringiensis* Cry4A toxin reveals a putative disulphide bond in the loop connecting helices 4 and 5 that may play a role in function of the toxin. In this study, the recombinant plasmid harboring the *cry4A* gene under control of the *tac* promoter together with the *cry4B* regulatory region was constructed and expressed in *Escherichia coli.* Upon solubilization and trypsin digestion, the 130-kDa Cry4A protein was processed into a 47-kDa polypeptide and a ca. 20-kDa fragment composed of α1-α5. SDS-PAGE showed that the 20-kDa fragment treated with β-mercaptoethanol had mobility slower than the untreated protein, indicating the existence of the C192-C199 disulphide bond within the loop connecting α4 and α5 of the Cry4A toxin. To investigate the role in toxicity of this disulphide bond, site-directed mutagenesis was employed to convert either Cys-192 or Cys-199 to alanine in order to eliminate the disulphide bond. Like the wild-type protein, the non-disulphide bridged mutants were highly expressed as inclusion bodies and were structurally stable upon solubilization and trypsin activation. Gel-shift assays have confirmed disappearance of the pre-existent disulphide bond. The larvicidal activity against *Aedes aegypti* of *E. coli* cells expressing either C192A or C199A mutant toxin has approximately the same as the wild-type toxin. Interestingly, the larvicidal activity of the wild-type inclusions were apparently at least 2-fold more toxic than both mutant inclusions, thus suggesting that the disulphide bond within the α4-α5 loop might indeed be involved in the Cry4A toxin mechanism.