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ISSARA SRAMALA PROLINE SCANNING MUTAGENESIS OF SELECTED HELICES OF THE *Bacillus thuringiensis* Cry4B TOXIN. THESIS ADVISORS : CHANAN ANGSUTHANASOMBAT Ph.D., CHARTCHAI KRITTANAI, Ph.D., GERD KATZENMEIER Ph.D. 99 p. ISBN 974-662-707-4

The proposed mode of action of *Bacillus thuringiensis* (*Bt*) δ-endotoxins is the formation of lytic pores in the susceptible larval midgut epithelium. Recently published X-ray structures of two different Cry δ-endotoxins (Cry1Aa and Cry3A) revealed a possible apparatus for pore-formation in the form of a seven-amphipathic helix bundle in which five helices (α3, α4, α5, α6 and α7) are potential candidates for inserting into the membrane to form a transmembrane pore.

In this study, PCR-based mutagenesis was employed to investigate the role for toxicity of the putative transmembrane helices of the 130-kDa Cry4B mosquito-larvicidal toxin produced by *Bt* subsp *israelensis*. Mutant toxins with a single proline substitution in the central region of α5, α6 and α7 were constructed on the basis of the homology based-Cry4B model, and were over-expressed in *Escherichia coli* as cytoplasmic inclusion bodies with a yield similar to that of the wild-type toxin. Unlike the wild-type inclusion, all proline-substituted inclusions were insoluble in carbonate buffer, pH 9.0, and gave no stable products when the solubilising buffer was supplemented with trypsin. A complete loss of larvicidal activity against *Aedes aegypti* larvae was also demonstrated for *E. coli* cells expressing mutant toxins in which residues Ala-182 or Leu-186 in α5, Tyr-220 or Ile-221 in α6, or Thr-254 or Val-257 in α7 were mutated. The results therefore suggest that these six residues might be involved in solubility and proteolytic susceptibility or toxicity of the Cry4B toxin.